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(54) Title: HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND THERAPEUTIC AND PROPHYLACTIC USES THEREOF		
(57) Abstract The invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein, vaccines comprising the mutant HIV-1 envelope glycoprotein, antibodies and methods of treating individuals.		

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5 HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND
THERAPEUTIC AND PROPHYLACTIC USES THEREOF

10 Background of the Invention

10 Throughout this application, various publications are
referenced by Arabic numerals. Full citations for these
references may be found at the end of the specification
immediately preceding the claims. The disclosure of these
15 publications is hereby incorporated by reference into this
application to describe more fully the art to which this
invention pertains.

20 The life cycle of animal viruses is characterized by a
series of events that are required for the productive
infection of the host cell. The initial step in the
replicative cycle is the attachment of the virus to the cell
surface, which attachment is mediated by the specific
interaction of the viral attachment protein (VAP) to
25 receptors on the surface of the target cell. The
differential pattern of expression of these receptors is
largely responsible for the host range and tropic properties
of viruses. In addition, an effective immune response
against many viruses is mediated through neutralizing
30 antibodies directed against the VAP. The interaction of the
VAP with cellular receptors and the immune system therefore
plays a critical role in infection and pathogenesis of viral
disease.

35 The human immunodeficiency virus type 1 (HIV-1) infects
primarily helper T lymphocytes, dendritic cells, and
monocytes/macrophages--cells that express surface CD4--
leading to a gradual loss of immune function. This loss of
function results in the development of the human acquired

immunodeficiency syndrome (AIDS) (1). The initial phase of the HIV-1 replicative cycle involves the high-affinity interaction between the HIV-1 exterior envelope glycoprotein gp120 and cell surface CD4 (K_d approximately 4×10^{-9} M) (2).
5 Several lines of evidence demonstrate the requirement of this interaction for viral infectivity. The introduction into CD4⁺ human cells of cDNA encoding CD4 is sufficient to render otherwise resistant cells susceptible to HIV-1 infection (3). In vivo, viral infection appears to be
10 restricted to cells expressing CD4, indicating that the cellular tropism of HIV-1 is largely determined by the pattern of cellular expression of CD4. Following the binding of HIV-1 gp120 to cell surface CD4, viral and target cell membranes fuse by a mechanism that is poorly
15 understood, resulting in the introduction of the viral capsid into the target cell cytoplasm (4).

Mature CD4 has a relative molecular mass (M_r) of 55 kDa and consists of an N-terminal 372-amino acid extracellular
20 domain containing four tandem immunoglobulin-like regions (V1-V4), followed by a 23-amino acid transmembrane domain and a 38-amino acid cytoplasmic segment (5, 6). In experiments using truncated sCD4 proteins, it has been shown that the determinants for high-affinity binding to HIV-1
25 gp120 lie solely within the N-terminal immunoglobulin-like domain (V1) (7-9). Mutational analysis of V1 has defined a discrete binding site (residues 38-52) that comprises a region structurally homologous to the second complementarity-determining region (CDR2) of immunoglobulin
30 genes (9).

The production of large quantities of sCD4 has permitted a structural analysis of the two N-terminal immunoglobulin-like domains (V1V2). The structure determined at 2.3

angstrom resolution reveals that the molecule has two tightly-associated domains, each of which contains the immunoglobulin-fold connected by a continuous beta strand. The putative binding sites for monoclonal antibodies, class II major histocompatibility complex (MHC) molecules, and HIV-1 gp120, as determined by mutational analyses, map on the molecular surface (10, 11).

The HIV-1 envelope gene env encodes an envelope glycoprotein precursor, gp160, which is cleaved by cellular proteases before transport to the plasma membrane to yield gp120 and gp41. The membrane-spanning glycoprotein, gp41, is non-covalently associated with gp120, a purely extracellular glycoprotein. The mature gp120 molecule is heavily glycosylated (approximately 24 N-linked oligosaccharides), contains approximately 480 amino acid residues with 9 intra-chain disulfide bonds (12), and projects from the viral membrane as a dimeric or multimeric molecule (13).

Mutational studies of HIV-1 gp120 have delineated important functional regions of the molecule. The regions of gp120 that interact with gp41 map primarily to the N- and C-termini (14). The predominant strain-specific neutralizing epitope on gp120 is located in the 32-34 amino acid residue third variable loop, herein referred to as the V3 loop, which resides near the center of the gp120 sequence (15). The CD4 binding site maps to discontinuous regions of gp120 that include highly conserved or invariant amino acid residues in the second, third, and fourth conserved domains (the C2, C3, and C4 domains) of gp120 (16). It has been postulated that a small pocket formed by these conserved residues within gp120 could accommodate the CDR2 loop of CD4, a region defined by mutational analyses as important in interacting with gp120 (17).

HIV-1 gp120 not only mediates viral attachment to surface CD4 molecules, but also serves as the major target of antibodies which neutralize non-cell-associated virus and inhibit cell to cell viral transmission.

5

There are two major classifications of HIV-1-neutralizing antibodies: type-specific and group-common (15). Type-specific neutralizing antibodies primarily recognize linear determinants in the highly variable V3 loop of gp120. These
10 antibodies act by inhibiting fusion between HIV-1 and the target cell membrane, and generally neutralize only a particular isolate of, or closely related strains of, HIV-1. Sequence variation within the V3 loop, as well as outside of this region, permits viruses to escape neutralization by
15 anti-V3 loop antibodies. In contrast, group-common neutralizing antibodies primarily recognize discontinuous or conformational epitopes in gp120, and possess the ability to neutralize a diverse range of HIV-1 isolates. These broadly neutralizing antibodies often recognize a site on gp120
20 which overlaps the highly conserved CD4-binding site, and thus inhibits gp120-CD4 binding.

A structural relationship has been demonstrated between the V3 loop and the C4 region of gp120 which region constitutes
25 both part of the CD4 binding site and part of the conserved neutralization epitopes. It was observed that deleting the V3 loop resulted in significantly increased binding of a panel of broadly neutralizing hMoAbs (neutralizing human monoclonal antibodies) to the CD4 binding site (18).

30

A major goal in AIDS vaccine development is to develop a vaccine able to protect a subject against the numerous genetic variants of HIV-1 that infect humans. Although cell-mediated immune responses might serve to control
35 infection in HIV-1-infected individuals, several lines of

evidence demonstrate that protection against infection is mainly mediated by neutralizing antibodies directed against gp120. Early experiments showed that immunization of chimpanzees with recombinant gp120 induced a protective immune response against challenge with the homologous HIV-1 strain (17). This protection correlated with the presence of high-titer neutralizing antibodies against the V3 loop of gp120. In addition, passive immunization of chimpanzees with a V3-loop neutralizing monoclonal antibody resulted in protection against challenge with the homologous HIV-1 strain (19). Although protection against challenge was demonstrated in these two experiments, recent studies have questioned the clinical relevance of these findings. For example, these neutralizing antibodies recognize the V3 loop determinants of a single strain, and not conserved or discontinuous epitopes. Thus, these antibodies lack the ability to neutralize the broad spectrum of HIV-1 strains present in an HIV-1 population. Furthermore, the challenge virus was the homologous HIV-1 laboratory adapted LAI (HTLV-IIIB) strain and not one of the primary isolates that contain considerable gp120 sequence heterogeneity. Since these experiments showed that gp120 subunit vaccination induces an immune response effective against only the homogeneous HIV-1 strain used as an antigen, it is unlikely that the vaccination regimens used in these studies would be useful in humans.

Individuals infected by HIV-1 typically develop antibodies that neutralize the virus in vitro, and neutralization titers decrease with disease progression (19). Analysis of sera from HIV-1-infected humans indicates that type-specific neutralizing antibodies appear early in infection. Later in the course of infection, a more broadly neutralizing antibody response develops. However this antibody response is of significantly lower titer and/or affinity.

Fractionation studies of HIV-1 antibody-positive human sera reveal that the type-specific neutralizing activity is primarily directed against linear determinants in the V3 loop of gp120 (20). There was no correlation found among
5 antibodies between the ability to neutralize divergent HIV-1 isolates and reactivity to the V3 loop of these isolates. In contrast, the broadly neutralizing antibodies present in HIV-1 antibody-positive human sera primarily recognize discontinuous epitopes in gp120 which overlap the CD4-
10 binding site and block gp120-CD4 binding. In other words, the broadly neutralizing activity of neutralizing antibodies is not merely the result of additive anti-V3 loop reactivities against diverse HIV-1 isolates which appear during infection.

15 Recently, several groups have generated human monoclonal antibodies (hMoAbs) derived from HIV-1 infected individuals which possess type-specific or group-common neutralizing activities (17). The type-specific neutralizing hMoAbs were
20 found to recognize linear determinants in the V3 loop of gp120. In contrast, the group-common neutralizing hMoAbs generally recognize discontinuous epitopes which overlap the CD4-binding site and block gp120-CD4 binding.

25 The V3 loop is a highly immunodominant region of gp120 which partially interacts with the CD4-binding region. The presence of the V3 loop region on gp120 may skew the humoral immune response away from producing antibodies which specifically bind to the CD4-binding domain of gp120.
30 Furthermore, the advantages of removing the V3 loop to expose the CD4-binding domain of gp120 to the immune system would be countered by the fact that the exposed CD4-binding site would still have a high affinity for cell surface CD4. In other words, a mutant gp120 protein missing only the V3
35 loop would quickly bind to CD4+ cells and would thus be

hampered in generating an immune response against the exposed CD4-binding site.

The subject invention provides a mutant HIV-1 gp120 envelope glycoprotein which overcomes both the problems of V3 loop immunodominance and of the high affinity to CD4. The subject invention further provides vaccines comprising the mutant HIV-1 gp120 envelope glycoprotein, antibodies which specifically bind to the CD4-binding site of HIV-1 gp120 envelope glycoprotein, pharmaceutical compositions comprising these antibodies, and methods of using these vaccines and compositions to treat or prevent HIV-1 infection.

Summary of the Invention

The subject invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(W-X) point mutation, wherein X is an amino acid residue other than tryptophan. In the preferred embodiment, X is a valine residue.

10 In one embodiment, the nucleic acid molecule is a DNA molecule. The DNA molecule may be a plasmid. In one embodiment, the plasmid comprises the sequence of the plasmid designated PPI4-tPA.

15 In one embodiment, the C4 domain is an HIV-1_{LA1} gp120 envelope glycoprotein C4 domain. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{LA1} gp120 envelope glycoprotein.

20 In another embodiment, the C4 domain is an HIV-1_{JR-FL} gp120 envelope glycoprotein C4 domain. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein.

25 The subject invention also provides the mutant HIV-1 gp120 envelope glycoprotein encoded by the recombinant nucleic acid molecule of the subject invention.

The subject invention further provides a vaccine which
30 comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

The subject invention further provides a method of treating

an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of the subject invention, thereby treating the HIV-1-infected subject.

- 5 The subject invention further provides a vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.
- 10 The subject invention further provides a method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the HIV-1-exposed
- 15 subject's becoming infected with HIV-1.

- The subject invention further provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-
- 20 1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.

- The subject invention further provides a method of obtaining
- 25 partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed subject with the vaccine of the subject invention, (b) recovering from the immunized subject serum comprising said
- 30 antibodies, and (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein. In the preferred embodiment, the subject is a human.

In one embodiment, the subject is a medical practitioner.
In another embodiment, the subject is a newborn infant.

- 5 Finally, the subject invention provides a method of reducing
the likelihood of a non-HIV-1-exposed subject's becoming
infected with HIV-1 as a result of exposure thereto during
an incident wherein there is an increased risk of exposure
to HIV-1, which comprises administering to the subject
10 immediately prior to the incident a dose of the composition
of the subject invention effective to reduce the population
of HIV-1 to which the subject is exposed during the
incident, thereby reducing the likelihood of the subject's
becoming infected with HIV-1. In one embodiment, the
15 subject is a medical practitioner.

Brief Description of the FiguresFigure 1

gp120 structure. Shown is a box diagram of HIV-1 gp120 depicting the boundaries of the five constant domains (C1-C5) and the five variable domains (V1-V5). The amino acid residue numbering above the box begins at the initiator methionine found at the beginning of the signal sequence (S) and is approximated based on a consensus of all known HIV-1 gp120 amino acid sequences. Also shown are the C4 domain amino acid sequences of HIV-1 strains LAI and JR-FL. Above the C4 domain sequences are indicated two mutations that reduce gp120 binding to cell surface CD4; tryptophan to valine and aspartate to alanine.

15

Figure 2

PPI4-tPA-gp120_{LAI}. Expression vector with the HIV-1_{LAI} gp120 gene fused to the CMV MIE promoter, and the tPA signal sequence replacing the HIV-1 gp120 signal sequence. Abbreviations: CMV MIE = cytomegalovirus major immediate early, E = enhancer, P = promoter, EXA = Exon A, INA = Intron A, EXB = Exon B, tPA ss = human tissue plasminogen activator signal sequence, gp120 = glycoprotein 120, BGH = bovine growth hormone, AMP = ampicillin resistance gene, and DHFR = dihydrofolate reductase gene.

25

Figure 3

CMV MIE promoter fused to tPA-gp120_{LAI}. The nucleotide sequence of the CMV MIE promoter/enhancer region is shown fused to the HIV-1_{LAI} gp120 gene that contains the tPA signal sequence. The numbering of nucleotide sequence begins with the HincII site and the numbering of the amino acid sequence begins with the first methionine found in the tPA signal sequence. The tPA signal sequence is fused in-frame to Thr₃₁.

30

of gp120, the first amino acid found in mature gp120. The signal sequence is shown in bold as are various landmark restriction sites used for cloning as discussed in the text. The locations of Exon A, Intron A, Exon B and the transcription start site and the signal cleavage site are indicated.

Figure 4

Transient expression of gp120. Autoradiograph of ³⁵S-labeled supernatants from COS cell transfectants, immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. The plasmids used for transfection were: Lane 1: Mock transfected cells; lane 2: a vector encoding a CD4-immunoglobulin chimera as a positive transfection control; lane 3: PPI4-tPA-gp120_{LAI}; and lane 4: PPI4-tPA-gp120_{JR-FL}. Positions of molecular weight markers are indicated.

Figure 5

Determination of gp120 concentration by ELISA. Panel A: Concentrations of gp120 in media of CHO cell lines, stably transfected with PPI4-tPA-gp120_{LAI}, determined by ELISA. Panel B: A standard curve was established using known amounts of gp120.

Figure 6

Expression of gp120 in stably transfected CHO cells. Autoradiograph of ³⁵S-labeled supernatants from stable CHO cell lines, immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. Lane 1: clone 9; lane 2: clone 13; lane 3: clone 6; lane 4: Clone 5. Positions of molecular weight markers are indicated.

Figure 7

tPA-gp120_{JR-FL}. The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{JR-FL} gp120 is shown. The NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg₃₅ and Val₃₆ is indicated.

Figure 8

tPA-gp120_{LAI}-V3^(c). The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{LAI} gp120 with the V3 loop deleted and replaced with the pentapeptide TGAGH is shown. The V3 loop replacement and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg₃₅ and Thr₃₆ is indicated.

Figure 9

tPA-gp120_{JR-FL}-V3^(c). The nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{JR-FL} gp120 with the V3 loop deleted and replaced with the pentapeptide TGAGH is shown. The V3 loop replacement and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg₃₅ and Val₃₆ is indicated.

Figure 10

tPA-gp120_{LAI}-V3^(c)-CD4^(c). Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{LAI} gp120, with the V3 loop deleted and replaced with the pentapeptide TGAGH, and Trp₄₀₈ mutated to Val. The mutations and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg₃₅ and Thr₃₆ is indicated.

Figure 11

tPA-gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{JR-FL} gp120, with the V3 loop deleted and replaced with the pentapeptide TGAGH, and Trp₃₉₆ mutated to Val. The mutations and the NarI and NotI restriction endonuclease sites used for cloning are shown in bold. The predicted site of cleavage by signal peptidase between Arg₃₅ and Val₃₆ is indicated.

10

Figure 12

tPA-gp120_{LAI}-CD4⁽⁻⁾. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{LAI} gp120. The Trp₄₃₇ to Val CD4 binding mutation, the NarI and NotI restriction endonuclease sites used for cloning, and the predicted site of cleavage by signal peptidase between Arg₃₅ and Thr₃₆ are shown in bold.

Figure 13

20 tPA-gp120_{JR-FL}-CD4⁽⁻⁾. Shown is the nucleotide and deduced amino acid sequence of the tPA signal sequence fused to HIV-1_{JR-FL} gp120. The Trp₄₂₄ to Val CD4 binding mutation, the NarI and NotI restriction endonuclease sites used for cloning and the predicted cleavage by signal peptidase between Arg₃₅ and Val₃₆ are shown in bold.

25

Figure 14Expression of gp120 in stably transfected CHO cells.

Autoradiograph of super ³⁵S-labeled supernatants from stable CHO cell lines, immunoprecipitated with MoAb F105-Protein A-Sepharose complex, and run on a reducing 10% SDS-PAGE gel. Panel A: Lane 1: tPA-gp120_{LAI} CHO cells; lane 2: tPA-gp120_{LAI}-V3⁽⁻⁾ CHO cells; lane 3: tPA-gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾ CHO cells. Panel B: Lane 1: tPA-gp120_{JR-FL} CHO cells; lane 2: tPA-gp120_{JR-FL}-V3⁽⁻⁾

30

CHO cells; lane 3: tPA-gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾ CHO cells. Positions of molecular weight markers are indicated.

Figure 15

5 Purified gp120 proteins.

Silver stained 10% SDS-PAGE gel with a sample of purified gp120 proteins. Panel A: Lane 1: tPA-gp120_{LAI} CHO cells; lane 2: tPA-gp120_{LAI}-V3⁽⁻⁾ CHO cells; lane 3: tPA-gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾ CHO cells. Panel B: Lane 1: tPA-gp120_{JR-FL} CHO cells; lane 2: 10 tPA-gp120_{JR-FL}-V3⁽⁻⁾ CHO cells; lane 3: tPA-gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾ CHO cells. Positions of molecular weight markers are indicated.

Figure 16

15 Analysis of binding of recombinant mutant gp120 to cell surface human CD4 by FACS.

Plate 1. DG44 cells, a subclone of CHO cells which lack expression of the human CD4 protein, were used as control. Increasing concentrations of HIV-1 gp120_{LAI} did not show an increase in specific fluorescence when compared to 20 background. Plate 2. DG44 #3 cells are a CHO cell line transfected with the cDNA clone encoding the human CD4 protein. Increasing concentrations of HIV-1 gp120_{LAI} show a dramatic increase (or shift) in fluorescence. Plate 3. Similar to Plate 2 but the HIV-1 gp120_{LAI}-V3⁽⁻⁾ protein was 25 added. Again a large shift indicating binding to the DG44 #3 cells was seen. Plate 4. DG44 #3 cells were incubated with either HIV-1 gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾ protein or MoAb OKT4A an antibody with high affinity for human CD4. Only OKT4A bound to the cells.

Detailed Description of the Invention

The plasmids designated PPI4-tPA-gp120_{LAI} and PPI4-tPA-gp120_{JR-FL} were deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 under ATCC Accession Nos. 75431 and 75432, respectively. The plasmids PPI4-tPA-gp120_{LAI} and PPI4-tPA-gp120_{JR-FL} were deposited with the ATCC on March 12, 1993.

The subject invention provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(w->x) point mutation, wherein X is an amino acid residue other than tryptophan. In the preferred embodiment, X is a valine residue.

In one embodiment, the nucleic acid molecule is a DNA molecule. The DNA molecule may be a plasmid. In one embodiment, the plasmid comprises the sequence of the plasmid designated PPI4-tPA.

The V3 loop of HIV-1 gp120 envelope glycoprotein is shown in Figure 1. The V3 loop is demarcated by cysteine residues at both its N- and C-termini. As used herein, a V3 loop deletion means a deletion of one or more amino acid residues between the terminal cysteine residues, with the proviso that there must be three or more amino acid residues situated between the two terminal cysteine residues in a V3 loop deletion. These three or more amino acid residues may either be residues originally present in the V3 loop, or exogenous residues. For example, as shown in the

Experimental Details section *infra*, the pentapeptide TGAGH is situated between the two terminal cysteine residues. Variations in the size of the V3 loop deletion illustrated herein are tolerable without affecting the overall structure of the mutant HIV-1 gp120 envelope glycoprotein, as is well known to those skilled in the art.

As used herein, "C4 domain" means the HIV-1 gp120 envelope glycoprotein C4 domain having the following consensus sequence:

X₁X₂X₃CX₄IX₅X₆X₇X₈X₉X₁₀**W**X₁₁X₁₂X₁₃X₁₄X₁₅AX₁₆YX₁₇X₁₈-
PX₁₉X₂₀X₂₁X₂₂X₂₃X₂₄X₂₅X₂₆SX₂₇X₂₈TGX₂₉X₃₀X₃₁X₃₂RX₃₃GX₃₄,

wherein X₁ = T, I, V, K or R; X₂ = L, I or H; X₃ = P, Q, L or T; X₄ = R, K or G; X₅ = K or E; X₆ = Q or E; X₇ = F, I or V; X₈ = I, V or M; X₉ = N, R or K; X₁₀ = M, R, L or T; X₁₁ = Q, R or V; X₁₂ = E, K, G, R, V or A; X₁₃ = V, T, A or G; X₁₄ = G or E; X₁₅ = K, R, E, or Q; X₁₆ = M, V, I or L; X₁₇ = A, T or D; X₁₈ = P or L; X₁₉ = I or F; X₂₀ = S, R, G, K, N, A, E or Q; X₂₁ = G or R; X₂₂ = Q, L, P, N, K, V, T, E or I; X₂₃ = I, V or L; X₂₄ = R, K, S, N, G, I, T, E or I; X₂₅ = C or R; X₂₆ = S, L, I, T, P, E, V, K, D or N; X₂₇ = N, K or L; X₂₈ = I or V; X₂₉ = L, P or I; X₃₀ = L or I; X₃₁ = L or I; X₃₂ = T, A, I, V or E; X₃₃ = D or E; X₃₄ = G or V.

The C4 domain consensus sequence is based on existing C4 domain sequence information from various HIV-1 strains, and thus is not necessarily an exhaustive consensus sequence. The conserved tryptophan residue shown in bold after residue X₁₀ is the only conserved tryptophan residue in the C4 domain. As used herein, a C4 domain_(W->X) point mutation is a mutation of the above-identified conserved C4 domain tryptophan residue to an amino acid residue other than

tryptophan. For example, a C4 domain_(W->V) point mutation is a mutation of the conserved C4 domain tryptophan residue to a valine residue.

5 In one embodiment, the C4 domain is an HIV-1_{LAI} gp120 envelope glycoprotein C4 domain. The sequence of the HIV-1_{LAI} gp120 C4 domain is: TLPCRKQFINMWQEVGKAMYAPPISGQIRCS-SNITGLLLTRDGG. The mutant HIV-1 gp120 envelope glycoprotein may be a mutant HIV-1_{LAI} gp120 envelope glycoprotein.

10

In another embodiment, the C4 domain is an HIV-1_{JR-FL} gp120 envelope glycoprotein C4 domain. The sequence of the HIV-1_{JR-FL} gp120 C4 domain is: 'TLPCRKQIINMWQEVGKAMYAPPIRGQIRCS-SNITGLLLTRDGG. The mutant HIV-1 gp120 envelope glycoprotein
15 may be a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein.

HIV-1_{LAI} is a laboratory-adapted strain that is tropic for phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes (PBLs) and immortalized human T-cell lines. In
20 contrast, HIV-1_{JR-FL} was isolated from brain tissue taken at autopsy that was co-cultured with lectin-activated normal human PBLs. HIV-1_{JR-FL} is tropic for PHA-stimulated PBLs and blood-derived macrophages but will not replicate in transformed T-cell lines. Mutant HIV-1 gp120 envelope
25 glycoproteins derived from a clinical isolate of HIV-1 such as JR-FL may possess new or different epitopes compared to the laboratory-adapted HIV-1 strains that are beneficial for successful vaccination. Although only the HIV-1_{LAI} and HIV-1_{JR-FL} strains are used herein to generate the mutant HIV-1
30 gp120 envelope glycoproteins of the subject invention, other HIV-1 strain could be substituted in their place as is well known to those skilled in the art.

The V1 and V2 variable regions of gp120 are unnecessary for

CD4 binding (21). Therefore the mutant HIV-1 gp120 envelope glycoprotein of this invention can either include or exclude the V1 and V2 variable regions.

- 5 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(Asp→X) point mutation, wherein the aspartate residue is between amino acid residues X₁₅ and X₁₆ in the C4 consensus
10 sequence, and X is an amino acid residue other than aspartate or glutamate. In the preferred embodiment, X is an alanine residue.

- The subject invention additionally provides a recombinant
15 nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(Glu→X) point mutation, wherein the glutamate residue is between amino acid residues X₁₅ and X₁₆ in the C4 consensus sequence, and X is an amino acid residue other than
20 aspartate or glutamate. In the preferred embodiment, X is an alanine residue.

- The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LAI} gp120
25 envelope glycoprotein comprising a V3 loop deletion and a C3 domain_(asp378→X) point mutation, wherein X is an amino acid residue other than aspartate or glutamate. In the preferred embodiment, X is a lysine residue.

- 30 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 domain_(asp369→X) point mutation, wherein X is an amino acid residue other than aspartate or glutamate. In the preferred

embodiment, X is a lysine residue.

The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LA1} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 domain_(glu380->X) point mutation, wherein X is an amino acid residue other than glutamate. In the preferred embodiment, X is a glutamine residue.

- 10 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein comprising a V3 loop deletion and a C3 domain_(glu371->X) point mutation, wherein X is an amino acid residue other than glutamate. In the preferred embodiment, X is a glutamine residue.

- 20 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{LA1} gp120 envelope glycoprotein comprising a V3 loop deletion and a C2 domain_(thr267->X) point mutation, wherein X is an amino acid residue other than threonine. In the preferred embodiment, X is an arginine residue.

- 25 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein comprising a V3 loop deletion and a C2 domain_(thr260->X) point mutation, wherein X is an amino acid residue other than threonine. In the preferred embodiment, X is an arginine residue.

- 30 The subject invention additionally provides a recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising (a) a V3 loop deletion, or (b) a one of the C2, C3 or C4 domain point mutations

discussed supra.

The point mutations in the recombinant nucleic acid molecules described supra are selected based on their
5 ability to reduce the affinity of the mutant gp120 glycoprotein encoded thereby for CD4. As used herein, the term "reduce the affinity" means to reduce the affinity by at least two-fold.

10 One skilled in the art would know how to make recombinant nucleic acid molecules which encode mutant HIV-1 gp120 envelope glycoproteins comprising a V3 loop deletion and the specific C2, C3 or C4 domain point mutations corresponding to those mutations exemplified in the HIV-1_{JR-FL} and HIV-1_{LAI}
15 strains, supra. Furthermore, one skilled in the art would know how to use these recombinant nucleic acid molecules to obtain the proteins encoded thereby, and practice the therapeutic and prophylactic methods of using same, as described herein for the recombinant nucleic acid molecule
20 which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(W->X) point mutation.

The subject invention also provides the mutant HIV-1 gp120
25 envelope glycoprotein encoded by the recombinant nucleic acid molecule of the subject invention.

In accordance with the invention, numerous vector systems for expression of the mutant HIV-1 gp120 envelope
30 glycoprotein may be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MoMLV), Semliki Forest virus or SV40 virus.

Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototrophy to an auxotrophic host, biocide resistance, (e.g., antibiotics) or resistance to heavy metals such as copper or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements include those described by Okayama (22).

The vectors used in the subject invention are designed to express high levels of mutant HIV-1 gp120 envelope glycoproteins in cultured eukaryotic cells as well as efficiently secrete these proteins into the culture medium. The targeting of the mutant HIV-1 gp120 envelope glycoproteins into the culture medium is accomplished by fusing in-frame to the mature N-terminus of the mutant HIV-1 gp120 envelope glycoprotein the tissue plasminogen activator (tPA) prepro-signal sequence.

The mutant HIV-1 gp120 envelope glycoprotein may be produced by a) transfecting a mammalian cell with an expression vector for producing mutant HIV-1 gp120 envelope glycoprotein; b) culturing the resulting transfected mammalian cell under conditions such that mutant HIV-1 gp120 envelope glycoprotein is produced; and c) recovering the mutant HIV-1 gp120 envelope glycoprotein so produced.

Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression

vectors may be transfected or introduced into an appropriate mammalian cell host. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation or other
5 conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity. Expression of the gene encoding a mutant HIV-1 gp120 envelope glycoprotein results in production of the mutant glycoprotein.

10 Methods and conditions for culturing the resulting transfected cells and for recovering the mutant HIV-1 gp120 envelope glycoprotein so produced are well known to those skilled in the art, and may be varied or optimized depending
15 upon the specific expression vector and mammalian host cell employed.

In accordance with the claimed invention, the preferred host cells for expressing the mutant HIV-1 gp120 envelope glycoprotein of this invention are mammalian cell lines. Mammalian cell lines include, for example, monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line 293; baby hamster kidney cells (BHK); Chinese hamster ovary-cells-DHFR (CHO); Chinese hamster ovary-cells DHFR
25 (DXB11); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); mouse cell line (C127); and myeloma cell lines.

30 Other eukaryotic expression systems utilizing non-mammalian vector/cell line combinations can be used to produce the mutant HIV-1 gp120 envelope glycoproteins. These include, but are not limited to, baculovirus vector/insect cell

expression systems and yeast shuttle vector/yeast cell expression systems.

5 Methods and conditions for purifying mutant HIV-1 gp120 envelope glycoproteins from the culture media are provided in the invention, but it should be recognized that these procedures can be varied or optimized as is well known to those skilled in the art.

10 The subject invention further provides a vaccine which comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

15 A therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein may be determined according to methods well known to those skilled in the art.

20 As used herein, adjuvants include, but are not limited to, alum, Freund's incomplete adjuvant (FIA), Saponin, Quil A, Monophosphoryl lipid A (MPL), and nonionic block copolymers (SAF) such as L-121 (Pluronic; Syntex SAF). In the preferred embodiment, the adjuvant is alum, especially in the form of a thixotropic, viscous, and homogeneous aluminum hydroxide
25 gel. The vaccine of the subject invention may be administered as an oil in water emulsion. Methods of combining adjuvants with antigens are well known to those skilled in the art.

30 The subject invention further provides a method of treating an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of the subject invention, thereby treating the HIV-1-infected subject.

35 As used herein, treating an HIV-1-infected subject with the

vaccine of the subject invention means reducing in the subject either the population of HIV-1 or HIV-1-infected cells, or ameliorating the progression of an HIV-1-related disorder in the subject.

5

As used herein, an "HIV-infected subject" means an individual having at least one of his own cells invaded by HIV-1.

10 As used herein, "immunizing" means administering a primary dose of the vaccine to a subject, followed after a suitable period of time by one or more subsequent administrations of the vaccine, so as to generate in the subject an immune response against the CD4-binding region of the mutant HIV-1
15 gp120 envelope glycoprotein in the vaccine. A suitable period of time between administrations of the vaccine may readily be determined by one skilled in the art, and is usually in the order of several weeks to months.

20 In the preferred embodiment, the dose of vaccine administered is an amount sufficient to deliver to the subject between 10ug and 1mg of the mutant HIV-1 gp120 envelope glycoprotein.

25 The subject invention further provides a vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of the subject invention, and an adjuvant.

30 A prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein may be determined according to methods well known to those skilled in the art.

The subject invention further provides a method of reducing
35 the likelihood of an HIV-1-exposed subject's becoming

infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the HIV-1-exposed subject's becoming infected with HIV-1.

5

As used herein, the subject's becoming infected with HIV-1 means the invasion of the subject's own cells by HIV-1.

As used herein, reducing the likelihood of a subject's becoming infected with HIV-1 means reducing the likelihood of the subject's becoming infected with HIV-1 by at least two-fold. For example, if a subject has a 1% chance of becoming infected with HIV-1, a two-fold reduction in the likelihood of the subject's becoming infected with HIV-1 would result in the subject's having a 0.5% chance of becoming infected with HIV-1. In the preferred embodiment of this invention, reducing the likelihood of the subject's becoming infected with HIV-1 means reducing the likelihood of the subject's becoming infected with HIV-1 by at least ten-fold.

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As used herein, an HIV-1-exposed subject is a subject who has HIV-1 present in his body, but has not yet become HIV-1-infected.

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The subject invention further provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-1-exposed subject with the vaccine of the subject invention, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.

30

As used herein, a non-HIV-1-exposed subject is a subject who does not have HIV-1 present in his body.

35

The subject invention further provides a method of obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed
5 subject with the vaccine of the subject invention, (b) recovering from the immunized subject serum comprising said antibodies, and (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120
10 envelope glycoprotein. In the preferred embodiment, the subject is a human.

As used herein, partially purified antibodies means a composition which comprises antibodies which specifically
15 bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein, and consists of fewer protein impurities than does the serum from which the anti-CD4-binding domain antibodies are derived. A protein impurity means a protein other than the anti-CD4-binding domain antibodies. For
20 example, the partially purified antibodies might be an IgG preparation.

Methods of recovering serum from a subject are well known to those skilled in the art. Methods of partially purifying
25 antibodies are also well known to those skilled in the art, and include, by way of example, filtration, ion exchange chromatography, and precipitation.

In one embodiment, the partially purified antibodies
30 comprise an immune globulin (IG) preparation. IG can be purified from serum by a two-step process. Initially, serum is fractionated by the cold ethanol method of Cohn, et al. (29). Cohn Fraction II has as its main protein component IgG immunoglobulin present as monomers, dimers and
35 aggregates. Fraction II is then purified to produce IVIG

(immune globulin intravenous) using a variety of purification methods which include, for example, ion exchange, DEAE chromatography, acid pH 4.25 diafiltration, PEG precipitation or Pepsin treatment. The final product is
5 stabilized (e.g., glucose + NaCl) and the final IgG concentration is fixed at between about 3% and about 6%.

The subject invention further provides the partially purified antibodies produced by the method of the subject
10 invention.

The subject invention further provides a pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of the subject
15 invention, and a pharmaceutically acceptable carrier.

A therapeutically effective amount of the partially purified antibodies of the subject invention may be determined according to methods well known to those skilled in the art.
20

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable
25 carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include
30 water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid
35 and nutrient replenishers, electrolyte replenishers such as

(immune globulin intravenous) using a variety of purification methods which include, for example, ion exchange, DEAE chromatography, acid pH 4.25 diafiltration, PEG precipitation or Pepsin treatment. The final product is
5 stabilized (e.g., glucose + NaCl) and the final IgG concentration is fixed at between about 3% and about 6%.

The subject invention further provides the partially purified antibodies produced by the method of the subject
10 invention.

The subject invention further provides a pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of the subject
15 invention, and a pharmaceutically acceptable carrier.

A therapeutically effective amount of the partially purified antibodies of the subject invention may be determined according to methods well known to those skilled in the art.

20

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable
25 carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include
30 water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid
35 and nutrient replenishers, electrolyte replenishers such as

those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

5

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

As used herein, administering may be effected or performed using any of the various methods known to those skilled in the art. The administering may comprise administering intravenously. The administering may also comprise administering intramuscularly. The administering may further comprise administering subcutaneously.

The dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10 mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

30

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-

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those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

5

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

As used herein, administering may be effected or performed using any of the various methods known to those skilled in the art. The administering may comprise administering intravenously. The administering may also comprise administering intramuscularly. The administering may further comprise administering subcutaneously.

The dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10 mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

30

The subject invention further provides a method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-

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infected subject.

The dose of the pharmaceutical composition of the subject invention effective to reduce the population of HIV-1 in the
5 HIV-1-infected subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10 mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred
10 embodiment, the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

The subject invention further provides a composition which
15 comprises a prophylactically effective amount of the partially purified antibodies of the subject invention, and a pharmaceutically acceptable carrier.

A prophylactically effective amount of the partially
20 purified antibodies of the subject invention may be determined according to methods well known to those skilled in the art.

The subject invention further provides a method of reducing
25 the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises administering to the HIV-1-exposed subject a dose of the composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-exposed subject, thereby reducing the
30 likelihood of the subject's becoming infected with HIV-1.

In one embodiment, the subject is a medical practitioner. The medical practitioner may be a medical practitioner exposed to an HIV-1-containing bodily fluid. As used herein,
35 the term "medical practitioner" includes, but is in no way

limited to, doctors, dentists, surgeons, nurses, medical laboratory assistants, and students in health care programs.

In another embodiment, the subject is a newborn infant. The
5 newborn infant may be a newborn infant born to an HIV-1-infected mother.

The dose of the composition of the subject invention effective to reduce the population of HIV-1 in the HIV-1-
10 exposed subject may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10mg/kg and 150mg/kg of protein if administered intramuscularly. In the preferred embodiment,
15 the dose is sufficient to deliver to the subject between about 100 mg/kg and 2g/kg of protein if administered intravenously.

The vaccines and pharmaceutical compositions of the subject
20 invention may also ameliorate the progression of an HIV-1-related disorder in a subject to whom the vaccines or pharmaceutical compositions were administered while the subject was either non-HIV-1-exposed or HIV-1-exposed, but not yet HIV-1-infected.

25 Finally, the subject invention provides a method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1 as a result of exposure thereto during an incident wherein there is an increased risk of exposure
30 to HIV-1, which comprises administering to the subject immediately prior to the incident a dose of the composition of the subject invention effective to reduce the population of HIV-1 to which the subject is exposed during the incident, thereby reducing the likelihood of the subject's
35 becoming infected with HIV-1. In one embodiment, the

subject is a medical practitioner.

An incident wherein there is an increased risk of exposure to HIV-1 includes, for example, receiving a blood
5 transfusion, sexual contact with an HIV-1-infected individual, and performing a HIV-1-containing bodily fluid-exposing medical procedure.

As used herein, "immediately prior to the incident" means
10 within one month of the incident. In the preferred embodiment, "immediately prior to the incident" means within one day of the incident.

The dose of the composition of the subject invention
15 effective to reduce the population of HIV-1 to which the subject is exposed during the incident may be readily determined using methods well known to those skilled in the art. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 10mg/kg and 150mg/kg of
20 protein if administered intramuscularly. In the preferred embodiment, the dose is sufficient to deliver to the subject between about 100mg/kg and 2g/kg of protein if administered intravenously.

25 One embodiment of this invention is a method of substantially reducing the likelihood of a non-infected medical practitioner's becoming infected with HIV-1 during a bodily fluid-exposing medical procedure involving a patient, which comprises administering to the patient during
30 a suitable time period an amount of the composition of the subject invention effective to substantially reduce the likelihood of the non-infected medical practitioner's becoming infected with HIV-1 by virtue of contact with the patient's bodily fluid during the medical procedure.

As used herein, a bodily fluid is any fluid which is present in the human body and is capable of containing infectious HIV-1 in an HIV-1-infected patient. Bodily fluids include, but are not limited to, saliva, cerebrospinal fluid, tears, 5 vaginal secretions, urine, alveolar fluid, synovial fluid and pleural fluid.

Another embodiment of this invention is a method of substantially reducing the likelihood of a non-HIV-1- 10 infected newborn infant's becoming infected with HIV-1 prior to or during birth from an HIV-1-infected mother, which comprises administering to the mother prior to birth an amount of the composition of the subject invention effective to substantially reduce the likelihood of the non-HIV-1- 15 infected newborn infant's becoming infected with HIV-1 by virtue of contact with the patient's bodily fluid.

In order to facilitate an understanding of the Experimental Details section which follows, certain frequently occurring 20 methods and/or terms are best described in Maniatis et al. (23).

This invention will be better understood by reference to the Experimental Details which follow, but those skilled in the 25 art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Nomenclature

As used herein, V3⁽⁻⁾ indicates a V3 loop deletion from HIV-1 gp120 envelope glycoprotein. As used herein, CD4⁽⁻⁾ indicates a point mutation in the C4 domain of HIV-1 gp120 envelope glycoprotein which mutation inhibits CD4 binding to the mutant HIV-1 gp120 envelope glycoprotein. The structure of HIV-1 gp120 envelope glycoprotein is shown in Figure 1.

Materials and Methods

1. Construction of PPI4-tPA-gp120_{LAI} expression vector.

An expression vector was constructed that consisted of the cytomegalovirus major immediate early (CMV MIE) promoter/enhancer linked to the HIV-1_{LAI} env gene, which gene had its signal sequence replaced by the tPA signal sequence. The CMV MIE promoter/enhancer sequences were derived from pSVCC1 (24) consisting of 1580 base pairs of contiguous DNA that is immediately 5' to the initiator ATG. In sequential order, the functional domains of the CMV promoter are: the promoter/enhancer region; a transcriptional initiator site; exon A (a non-coding exon); intron A; and 17 nucleotides of exon B (non-coding sequences). The viral promoter sequences were ligated to a gene construct consisting of the nucleotide sequences encoding amino acids -35 to -1 of human tPA (25) fused in-frame to HIV-1_{LAI} env amino acids 31 through 515, ending with a TGA stop codon. The construction was performed in two parts. The majority of the CMV promoter could be isolated as a 1560 bp Hinc II/Pst I fragment which was ligated to a Pst I/Not I 1590 bp DNA fragment that contained the remainder of the CMV promoter, the initiator ATG, the tPA signal sequence and the mature HIV-1_{LAI} env protein coding sequence.

The latter fragment was assembled using the polymerase chain reaction as follows. Primer 1 (GATCCTGCAGTCACCGTCCTTGACACGATGGATGCAATGAAGAGA) and primer 2 (AAGTCTTCTCCTCGGTCTTGTCTTTTAAACACCCAG) were used to amplify the nucleic acid sequences encoding the tPA signal sequence amino acids -35 to -1 from plasmid pMAM neo-s (Clonetech), thus producing a 150 bp fragment. A second 1440 bp DNA fragment was amplified using primer 3 (TTCAGAAGAGGAGCCAGAACAGAAAAATTGTGGGTC), primer 4 (GGAAAAAAGCGGCCGCTCATTTTTCTCTCTGCACCACTC), and pENV (26) as a template. The PCR fragments were pooled, desalted, and excess primer removed by ultrafiltration through a centricon-100 unit (Amicon). An aliquot of the pooled material was then subjected to a second round of amplification in the presence of primers 1 and 4 to produce a 1590 bp fragment, which was then digested with Pst I and Not I. The CMV promoter fragment and the HIV-1_{LAI} env fragment were then ligated together, and the entire transcription unit subcloned into PPI4, which is a eukaryotic shuttle vector that contains an ampicillin resistance gene, an SV40 origin of replication and a DHFR gene whose transcription is driven by the β -globin promoter. The final construct, PPI4-tPA-gp120_{LAI}, is shown in Figure 2.

The expression vector is then used as the prototype vector for the expression of gp120 proteins that are derived from other HIV-1 strains or mutated as described in the methods section. The vector was constructed so that unique Nar I and Not I sites flank the gp120 sequence, thus facilitating the removal of the gp120 gene cassette and the subsequent insertion of other gene cassettes (Figure 2).

2. Expression of HIV-1_{LAI} gp120 in mammalian cells.

a. Transient expression.

CosM5 cells grown in DMEM containing 10% fetal calf serum

were split to 75% confluence. On the following day, the cells were transfected for 16-20 hours with 10 micrograms of CsCl-purified PPI4-tPA-gp120_{LAI} DNA by the standard CaPO₄ (5) precipitation technique. After transfection, fresh medium was added to the cells. Analysis of the products synthesized 96-120 hours post-transfection was performed by radiolabelling the transfectants with ³⁵S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed by SDS-PAGE under reducing conditions (Figure 4).

b. Stable expression.

Dhfr^r Chinese hamster ovary cells (CHO) were transfected with 20 micrograms of CsCl-purified DNA. Approximately 3-5 days post-transfection, cells were placed in selective medium (nucleoside-free alpha MEM containing 10% dialyzed fetal calf serum). Approximately 10-15 days post-selection, individual cell clones were picked. Media was analyzed for gp120 expression by radiolabelling the cells with ³⁵S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed in turn by SDS-PAGE under reducing conditions (Figure 6). The levels of gp120 in the media of these clones were also quantitated (Figure 5) by ELISA performed as follows. The method involves coating 96-well plates overnight with sheep polyclonal IgG against the highly conserved C-terminus of gp120 (D7234, Aalto Bioreagents). After washing, dilutions of a standard gp120 preparation in cell growth medium, or supernatant from the stably-transfected cells, were incubated for 1 hour. The plates were washed again, and incubated for one hour with a horseradish peroxidase-conjugated anti-gp120 monoclonal antibody (9204, DuPont). Following a final wash, the peroxidase substrate OPD (DuPont) was added and the amount

of gp120 determined by comparing absorbance of unknowns with a standard curve. Standards were prepared from purified gp120 made in CHO cells, a small quantity of which was obtained from Celltech Ltd. Clones expressing the highest
5 levels were subjected to successive rounds of amplification of the newly introduced DNA sequences in increasing concentrations of methotrexate. Stable CHO cell lines were thus generated which secrete at least 1 microgram/milliliter of HIV-1_{LAI} gp120.

10

3. Construction of PPI4-tPA-gp120_{JR-FL}

a. The HIV-1_{LAI} gp120 env nucleotide sequence in PPI4-tPA-gp120_{LAI} was replaced by the nucleotide sequence encoding the mature gp120_{JR-FL} protein. Using the polymerase chain
15 reaction, the JR-FL sequences were amplified from pUC112-1 (27) using primer 5 (GATCGGCGCCAGAGTAGAAAAGTTGTGGGTCAC) and primer 4. The PCR fragment was digested with the restriction endonucleases Nar I and Not I, and the fragment subcloned in between the Nar I and Not I sites in PPI4-tPA-
20 gp120_{LAI} to generate PPI4-tPA-gp120_{JR-FL} (Figure 7).

b. Transient expression.

CosM5 cells grown in DMEM containing 10% fetal calf serum were split to 75% confluence. On the following day, the
25 cells were transfected for 16-20 hours with 10 micrograms of CsCl-purified PPI4-tPA-gp120_{JR-FL} DNA by the standard CaPO₄ (5) precipitation technique. After transfection, fresh medium was added to the cells. Analysis of the products synthesized 96-120 hours post-transfection was performed by
30 radiolabelling the transfectants with ³⁵S-cysteine for 12-18 hours, followed by precipitation of media using a CD4-immunoglobulin-Protein A-Sepharose complex, followed by SDS-PAGE under reducing conditions (Figure 4).

4. Construction of PPI4-tPA-gp120_{LAI}-V3^(c).

The V3 loop in tPA-gp120_{LAI} consists of amino acids Cys₃₀₆ through Cys₃₃₃. In the V3^(c) mutant, the amino acids in between these cysteines are replaced by the pentapeptide sequence Thr-Gly-Ala-Gly-His. Using the Transformer Site-Directed Mutagenesis Kit (Clontech), the V3 loop sequence in PPI4-tPA-gp120_{LAI} is altered using the mutagenic primer 6 (CTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGC) and primer 7 (CTCGAGCATGCATTCGAAGCTCGCTGATC) as a selection primer. Primer 7 changes a unique Xba I site in the backbone of the parent PPI4 plasmid into a unique BstB I site. Briefly, the mutagenesis method requires incubating of the parent plasmid with the mutagenic primer and the selection primer, denaturing at 100°C for 3 minutes and then chilling on ice. In the presence of buffered deoxynucleotide triphosphates and T4 DNA polymerase, the primers are allowed to initiate the polymerization of one strand of plasmid DNA. T4 DNA ligase is used to seal the newly synthesized DNA strand to form a covalently closed circle. Hybrid plasmids are then transformed into a MutS strain of *E. coli* that is deficient in mismatch repair. After allowing for the growth of transformed cells, DNA is purified from the cells and digested with the selection restriction endonuclease, in this case Xba I. Parental plasmids are cleaved by Xba I while the mutant plasmid remains resistant to cleavage by virtue of the Xba I to BstB I conversion. Digested DNA is then used to transform *E. coli*, and colonies harboring the mutant plasmid are picked. Multiple mutagenic primers can be used in a single round of mutagenesis. The amino acid sequence of the modified protein is shown in Figure 8.

5. Construction of PPI4-tPA-gp120_{IR-FL}-V3^(c).

The V3 loop in tPA-gp120_{IR-FL} consists of amino acids Cys₂₉₃

through Cys₃₇₇. In the V3^(c) mutant, the amino acids in between these cysteines are replaced by the pentapeptide sequence Thr-Gly-Ala-Gly-His. Using the Transformer Site-Directed Mutagenesis Kit (Clonetech), the V3 loop sequence
5 in PPI4-tPA-gp120_{JR-FL} is altered using the mutagenic primer 6 (CTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGC) and primer 7 as a selection primer. The amino acid sequence of the modified protein is shown in Figure 9.

10 6. Construction of PPI4-tPA-gp120_{LAI}-CD4^(c).

Using the Transformer Site-Directed Mutagenesis Kit (Clonetech), the selection primer 7, and the mutagenic primer 8 (CAATTTATAAACATGGTGCAGGAAGTAGG), Trp₄₃₇ of tPA-gp120_{LAI}, which is in an equivalent position to the
15 tryptophan residue in the HXBc2 strain of HIV-1, is mutated to a Val in the expression vector PPI4-tPA-gp120_{LAI} to generate PPI4-tPA-gp120_{LAI}-CD4^(c). The sequence for gp120_{LAI}-CD4^(c) is shown in Figure 12.

20 7. Construction of PPI4-tPA-gp120_{JR-FL}-CD4^(c).

In a fashion similar to that described above, Trp₄₂₄ of tPA-gp120_{JR-FL} is mutated to a Val in the expression vector PPI4-tPA-gp120_{JR-FL} using the selection primer 7 and the mutagenic primer 9 (CAAATTATAAACATGGTGCAGGAAGTAGG) to generate PPI4-tPA-gp120_{JR-FL}-CD4^(c). The sequence for gp120_{JR-FL}-CD4^(c) is shown
25 in Figure 13.

8. Construction of PPI4-tPA-gp120_{LAI}-V3^(c)-CD4^(c).

The tPA-gp120_{LAI} double mutant, V3^(c)-CD4^(c), is constructed by
30 including the mutagenic primers 6 and 8, and the selection primer 7 simultaneously in the reaction tube with PPI4-tPA-gp120_{LAI} as the DNA template. The final construct is named PPI4-tPA-gp120_{LAI}-V3^(c)-CD4^(c), and its sequence is shown in figure 10.

9. Construction of PPI4-tPA-gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾.

The tPA-gp120_{JR-FL} double mutant, V3⁽⁻⁾-CD4⁽⁻⁾, is constructed by including the mutagenic primers 6 and 9, and the selection primer 7 simultaneously in the reaction tube with PPI4-tPA-gp120_{JR-FL} as the DNA template. The final construct is named PPI4-tPA-gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾, and its sequence is shown in figure 11.

10. Expression of mutant HIV-1 gp120 in mammalian cells.

10 a. Transient expression.

CosM5 cells grown in DMEM containing 10% fetal calf serum are split to 75% confluence. On the next day, the cells are transfected for 16-20 hours with 10 micrograms of CsCl-purified mutant HIV-1 DNA by the standard CaPO₄ (5) precipitation technique. After transfection, fresh medium is added to the cells. Analysis of the products synthesized 96-120 hours post-transfection is performed by radiolabelling the transfectants with ³⁵S-cysteine for 12-18 hours, followed by precipitation of media using a sheep polyclonal IgG against the highly conserved C-terminus of gp120.

b. Stable expression.

Dhfr^r Chinese hamster ovary cells (CHO) are transfected with 20 micrograms of CsCl-purified DNA encoding the native or mutant HIV-1 gp120 glycoproteins. Approximately 3-5 days post-transfection, cells are placed in selective medium (nucleoside-free alpha MEM containing 10% dialyzed fetal calf serum). Approximately 10-15 days post-selection, individual cell clones are picked. Media is analyzed for gp120 expression by radiolabelling the cells with ³⁵S-cysteine for 12-18 hours, followed by quantitative immunoprecipitation of media using a sheep polyclonal IgG against the highly conserved C-terminus of gp120, followed

in turn by SDS-PAGE under reducing conditions. Alternatively, one can quantitate the level of gp120 by ELISA performed as follows. The method involves coating 96-well plates overnight with sheep polyclonal IgG against the highly conserved C-terminus of gp120 (D7234, Aalto Bioreagents). After washing, dilutions of a standard gp120 preparation in cell growth medium, or supernatant from the stably-transfected cells, are incubated for 1 hour. The plates are washed again, and incubated for one hour with a human MoAb (F105, AIDS Research & Reference Reagent Program, No. 857). The plates are washed again, and incubated again for 1 hour with a horseradish-peroxidase-conjugated goat anti-human IgG (Cappel). Following a final wash, the peroxidase substrate OPD (DuPont) is added and the amount of gp120 determined by comparing absorbance of unknowns with a standard curve. Standards are prepared from purified gp120 made in CHO cells, a small quantity of which is obtained from Celltech Ltd. Clones expressing the highest levels are subjected to successive rounds of amplification of the newly introduced DNA sequences in increasing concentrations of methotrexate. Stable CHO cell lines are thus generated which secrete at least 1 microgram/milliliter of mutant HIV-1 gp120.

25 11. Purification of HIV-1 gp120 proteins.

A one-step immunoaffinity procedure is used to purify the recombinant gp120 molecules described. Briefly, culture supernatant is collected and clarified by centrifugation. An immunoaffinity column consisting of a matrix coupled to a sheep polyclonal anti-gp120 IgG (D7234, Aalto Bioreagents) directed against the highly conserved C-terminal end (APTKAKRRVVQREKR) of gp120 is used to specifically adsorb gp120 from the cell culture media. This antisera recognizes native gp120, the V3 loop deletion mutants, and the CD4⁽⁻⁾

mutants since the C-terminal ends of these molecules remain unaltered. The bound gp120 is then eluted with 2M MgCl₂, concentrated by Amicon filtration, and dialyzed into 10 mM HEPES, pH 7.0. The purity of the proteins is determined by
5 SDS-PAGE and silver staining.

12. Characterization of recombinant HIV-1 gp120 proteins.
The purified glycoproteins are subjected to extensive biochemical and immunologic characterization. The integrity
10 of the proteins is monitored by SDS-PAGE and silver staining under reducing and non-reducing conditions. The glycoproteins are deglycosylated by treatment with the enzyme N-glycosidase F which cleaves N-linked oligo-saccharides, and are assayed by SDS-PAGE and silver staining
15 to monitor molecular weight shifts. The purified glycoproteins are also tested for reactivity with several well characterized anti-gp120 monoclonal antibodies that recognize both linear and discontinuous epitopes. The binding affinity to sCD4 is estimated using an ELISA assay.

20 The purified proteins HIV-1 gp120_{LAI}, gp120_{LAI}-V3⁽⁻⁾, gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾, gp120_{JR-FL}, gp120_{JR-FL}-V3⁽⁻⁾, and gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾, were tested for their ability to bind cell surface human CD4. DG44 #3 cells, a recombinant cell line designed to express
25 human CD4 on the membrane surface, were grown in T flasks and trypsinized. 5 X 10⁵ cells/experiment were aliquoted into FACS buffer (PBS + 2% BSA and 0.1% NaN₃), washed several times in the same buffer, and then incubated with 100 ul of a solution of purified gp120 protein at 5ug/ml in
30 FACS buffer at 37°C for 2 hr. The cells were washed in FACS buffer, and then incubated in 100 ul solution containing 5ug/ml sheep polyclonal IgG against the highly conserved C-terminus of gp120 in FACS buffer at 37°C for 2 hr. The cells were washed in FACS buffer then incubated in 100 ul

solution containing FITC-labeled rabbit anti-sheep IgG polyclonal antibody at 37°C for 2 hr. The cells were washed with FACS buffer and then resuspended in 500 ul FACS buffer. The cells were then analyzed on a Becton Dickinson FACScan according to the manufacturer's instructions. As a control for expression of CD4 on the DG44 #3 cells, FITC-labeled OKT4A (Becton Dickinson) was used.

13. A protocol for inoculation of animals with the mutant HIV-1 gp120 envelope glycoproteins.

Alum is used as an adjuvant during the inoculation series. The inoculum is prepared by dissolving the mutant HIV-1 gp120 envelope glycoprotein antigen in physiologic saline at a final antigen concentration of 100 ug/ml. Preformed alum (aluminum hydroxide gel) is added to the solution to a final level of 500 ug/ml aluminum. The antigen is allowed to adsorb onto the alum gel for two hours at room temperature. Following adsorption, the gel with the antigen is washed twice with physiologic saline and resuspended in the saline to a protein concentration of 100 ug/ml.

Monkeys and/or Guinea Pigs are individually inoculated with four 100 ug doses of the mutant HIV-1 gp120 envelope glycoprotein antigen adsorbed onto alum. Each dose is injected intramuscularly. The doses are delivered one or five months apart (week 0, 4, 8 and 28). the animals are bled at intervals of two or four weeks. Serum samples are prepared from each bleed to assay for the development of specific antibodies as described in the subsequent sections.

30

14. Analysis of sera for anti-mutant HIV-1 gp120 envelope glycoprotein IgG antibodies.

Each serum sample is analyzed by ELISA. Polystyrene microtiter plates are coated with 0.5 ug per well of pure mutant HIV-1 gp120 envelope glycoprotein in phosphate-

35

buffered physiological saline (PBS) at 4°C. Each well is then washed with PBS containing 0.5% TWEEN-20 (PBS-TW). Test serum, diluted serially in PBS-TW, is added to the mutant HIV-1 gp120 envelope glycoprotein-containing wells and allowed to react with the adsorbed mutant HIV-1 gp120 envelope glycoprotein for one hour at 37°C. The wells are then washed extensively in PBS-TW. Each well then receives 0.1% p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8, containing 0.5 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. The ensuing reaction is allowed to proceed at room temperature for 30 minutes, at which time it is terminated by the addition of 3.0 N NaOH.

The greater the interaction of antibodies in the test serum with the mutant HIV-1 gp120 envelope glycoprotein, the greater is the amount of alkaline phosphatase bound onto the well. The phosphatase enzyme mediates the breakdown of p-nitrophenyl phosphate into a molecular substance which absorbs light at a wavelength of 405 nm. Hence, there exists a direct relationship between the absorbance at 405 nm of light at the end of the ELISA reaction and the amount of mutant HIV-1 gp120 envelope glycoprotein-bound antibody. All animals inoculated with mutant HIV-1 gp120 envelope glycoprotein whose serum reacts specifically with the mutant HIV-1 gp120 envelope glycoprotein in the ELISA have a positive antibody response against mutant HIV-1 gp120 envelope glycoprotein.

15. Analysis of sera for activity which specifically neutralizes HIV-1 infectivity.

30 Virus-neutralizing activity is determined with an assay based on the use of multiplicity curves in which the ratio of infectious virus surviving antibody treatment (V_a) is compared to infectious virus in uninhibited cultures (V_o) at various dilutions of antisera. The neutralization titer of

the sera is then interpolated as that sera dilution which yields one log reduction in infectious titer (i.e., $V_0/V_1 = 0.1$). Briefly, 4-fold dilutions of virus (laboratory-adapted and primary isolates) are prepared to yield
5 infectious doses of 0.1 to 100 TCID₅₀ (Tissue Culture Infection Dose) in 20 ul. Serial 3-fold dilutions of sera are also prepared and 20 ul of each serum dilution are incubated with each dilution of virus in duplicate for 60 minutes at room temperature in a 96-well microtiter plate.
10 20 ul of AA5 cells (PHA stimulated PBMCs for primary HIV-1 isolates) are then added to the serum/virus mixtures. Cells are cultured for 7 days by the addition of fresh medium every other day. On the seventh day, supernatant from each well is removed and tested for the presence of reverse
15 transcriptase (RT). Infection in each well is then scored as either positive or negative based on the RT counts, and the infectious dose of virus in each treatment group is calculated using the Reed and Muench (28) formula. The neutralization titers represent the reciprocal serum
20 dilution required to reduced infectious dose of virus by one log. The above culture time is for the prototypic HIV-1_{LAI} isolate tested on the AA5 cell line. In the case of primary isolates, the termination date is usually 11-14 days. Culture conditions for PBMCs is not as demanding since
25 doubling time is restricted. In the case of PBMCs, one day PHA stimulations are used at a final concentration of 1.5×10^6 /ml on day 0. Half that number of fresh PBMCs are then added again on days 4 and 8. This multiple addition of PBMCs is meant to amplify virus output upon successful
30 infection so that the readout RT signal is strong. Again, the final readout titer for the primary isolate/PBMC is the reciprocal serum dilution which reduces infectious titer by one log.

16. Passive hyperimmune therapy.

Non-HIV-1-infected humans are immunized with the mutant HIV-1 gp120 envelope glycoprotein antigens according to a protocol similar to that described above in section 12. For
5 passive hyperimmune therapy in HIV-1-infected individuals, blood plasma is taken from mutant HIV-1 gp120 envelope glycoprotein immunized, non-HIV-1-infected human donors whose plasma has high levels of neutralizing antibodies. The plasma is pooled from several donors, purified to remove
10 nonimmunoglobulin proteins and is then sterilized to kill any other viruses or pathogens. The treated plasma is then injected into individuals infected with HIV-1, with repeated injections every week, every two weeks, or every month.

Results

Eukaryotic expression vectors designed to express high levels of HIV-1_{LAI} gp120 and HIV-1_{JR-FL} gp120 were constructed.

5 The CMV MIE promoter/enhancer was used to drive the transcription of a gene fusion consisting of the human tPA signal sequence fused to mature gp120 (Figures 2 and 7). The complete sequence of the transcription unit from the Hinc II site of the CMV promoter/enhancer to the Not I site

10 just 3' from the stop codon in gp120 is shown in figure 3. This vector was used to transfect COSM5 cells in a transient assay. The transfected cells were labeled with ³⁵S-cysteine and the media immunoprecipitated with a CD4-immunoglobulin-Protein A-Sepharose complex. The precipitated products were

15 analyzed using a reducing 10% SDS-PAGE gel and autoradiography (Figure 4). A 120 kD band was detected when PPI4-tPA-gp120_{LAI} was used to transfect COS cells (lane 3). A band migrating with a slightly lower molecular mass was detected when PPI4-tPA-gp120_{JR-FL} was used to transfect COS

20 cells (lane 4). No radiolabeled products were detected in the mock infected cells. Using a sheep polyclonal antibody directed against the highly conserved C-terminal end of HIV-1 gp120 in an ELISA assay, the level of expression of HIV-1 gp120 was determined to be 2350 ng/ml.

25

The PPI4-tPA-gp120_{LAI} vector was then used to stably transfect the dhfr^r CHO cell line DXB11. Two days post-transfection, the cells were plated at low density in nucleoside-free medium. Eight days post-transfection,

30 surviving clones were isolated and expanded. Individual primary transfectants were tested for gp120 expression using the ELISA method described in the methods section. Several primary CHO transfectants expressed significant quantities (10-120 ng/ml) of gp120 (Figure 5). Three of the highest

expressing clones were then subjected to increasing concentrations of methotrexate in order to amplify, in tandem, the copy number of the dhfr and gp120 genes. Cell lines were established that express high levels of gp120 with rates of secretion greater than 1 mg/liter. These were then used to purify gp120 to homogeneity.

Six CHO cell lines were established, using the procedures described in the methods sections, that express high levels of the following proteins: HIV-1 gp120_{LAI}, gp120_{LAI}-V3⁽⁻⁾, gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾, gp120_{JR-FL}, gp120_{JR-FL}-V3⁽⁻⁾, and gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾. Metabolic labeling of these cells with ³⁵S-cysteine followed by immunoprecipitation with the human monoclonal antibody F105 and analyzed by SDS-PAGE and autoradiography showed the presence of the gp120 proteins in the culture supernatant (Figure 14). From these cell lines the gp120 proteins were purified to homogeneity. Analysis by SDS-PAGE followed by silver-staining showed the purity of these proteins to be greater than 90% (Figure 15).

It was shown by FACScan analysis that the two CD4 binding mutants HIV-1gp120_{LAI}-V3⁽⁻⁾-CD4⁽⁻⁾ and HIV-1 gp120_{JR-FL}-V3⁽⁻⁾-CD4⁽⁻⁾ had no appreciable binding to recombinant cell lines designed to express high levels of human CD4 on their membrane surface (Figure 16, panel 4 and data not shown, respectively).

Discussion

The advantage of using the mutant HIV-1 gp120 envelope glycoproteins as immunogens is that these proteins will not elicit an immune response against the V3 loop, a highly immunodominant epitope on gp120. This is significant because the V3 loop may skew the humoral immune response away from discontinuous epitopes in the CD4-binding site. Mutant HIV-1 gp120 envelope glycoproteins having partial and total V3 loop deletions have been made (30). Deletion of the V3 loop therefore exposes the CD4-binding site to the immune system, allowing the immune system to mount a response against this critical region (18). Another advantage of using the mutant HIV-1 gp120 envelope glycoprotein as an immunogen is that it has significantly reduced affinity for cell surface CD4. An efficient humoral immune response depends on the binding of antigen to B cell surface immunoglobulin. The presence of the high-affinity CD4 receptor on large numbers of cells in the body may significantly diminish the ability of native gp120 to induce an effective humoral immune response. The rationale of mutating gp120 at the CD4 binding site is to redirect the mutant HIV-1 gp120 envelope glycoprotein away from cell surface CD4 toward immunoglobulin-bearing B cells, thereby allowing the immune system to mount a response against, inter alia, the CD4-binding site.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Progenics Pharmaceuticals, Inc.
- (ii) TITLE OF INVENTION: HIV-1 VACCINES, ANTIBODY COMPOSITIONS RELATED THERETO, AND THERAPEUTIC AND PROPHYLACTIC USES THEREOF
- (iii) NUMBER OF SEQUENCES: 29
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Cooper & Dunham
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 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: USA
 - (F) ZIP: 10112
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.24
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
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- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/037,816
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- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) TELEX: 422523 COOPUI

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Cys Xaa Ile Xaa Xaa Xaa Xaa Xaa Trp Xaa Xaa Xaa
 1 5 10 15
 Xaa Xaa Ala Xaa Tyr Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 20 25 30
 Ser Xaa Xaa Thr Gly Xaa Xaa Xaa Xaa Arg Xaa Gly Xaa

54

35

40

45

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Thr Leu Pro Cys Arg Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val
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20           25           30
Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly
35           40           45

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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20           25           30
Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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45

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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36

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCAGAAGAG GAGCCAGAAC AGAAAAATTG TGGGTC

36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGAAAAAAGC GGCCGCTCAT TTTCTCTCT GCACCACTC

39

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGTAGAAAT TAATTGTACA GGTGCTGGAC ATTGTAACAT TAGTAGAGC

49

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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29

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAATTATATA ACATGGTGCA GGAAGTAGG

29

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAAATTATATA ACATGGTGCA GGAAGTAGG

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3125 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1555..3115
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTGACATTGA TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG	60
CCCATATATG GAGTTCCGCG TTACATAACT TACGGTAAAT GGCCCGCCTG GCTGACCGCC	120
CAACGACCCC CGCCCATGGA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG	180
GACTTTCCAT TGACGTCAT GGGTGGACTA TTTACGGTAA ACTGCCCACT TGGCAGTACA	240
TCAAGTGTAT CATATGCCAA GTACGCCCC TATTGACGTC AATGACGGTA AATGGCCCGC	300
CTGGCATTAT GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT	360
ATTAGTCATC GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA	420
GCGGTTTGAC TCACGGGGAT TTCCAAGTCT CCACCCCATT GACGTCAATG GGAGTTTGT	480
TTGGCACCAA AATCAACGGG ACTTTCCAAA ATGTCGTAAC AACTCCGCCC CATTGACGCA	540
AATGGGCGGT AGGCGGTGAC GGTGGGAGGT CTATATAAGC AGAGCTCGTT TAGTGAACCG	600
TCAGATCGCC TGGAGACGCC ATCCACGCTG TTTTGACCTC CATAGAAGAC ACCGGGACCG	660
ATCCAGCCTC CGCGGCGGG AACGGTGCAT TGGAACGCGG ATTCCCCGTG CCAAGAGTGA	720
CGTAAGTACC GCCTATAGAC TCTATAGGCA CACCCCTTTG GCTCTTATGC ATGCTATACT	780
GTTTTTGGCT TGGGCCAACA CCCCCTCCTA GATAGGTGAT GGTATAGCTT AGCCTATAGG	840
TGTGGGTTAT TGACCATTAT TGACCACTCC CCTATTGGTG ACGATACTTT CCATTACTAA	900
TCCATAACAT GGCCGCTCTT TGCCACAAC ATCTCTATTG GCTATATGCC AATACTCTGT	960
CCTTCAGAGA CTGACACGGA CTCTGTATTT TTACAGGATG GGGTCCCAT TATTATTTAC	1020
AAATTCACAT ATACAACAAC GCCGTCCCC GTGCCGCGAG TTTTATTAA CATGCGGGAT	1080
CTCCACGCGA ATCTCGGGTA CGTGTTCGG ACATGGGCTC TTCTCCGGTA GCGGCGGAGC	1140
TCCACATCCG AGCCTGTCCC ATGCCATGC CTCCAGCGGC TCATGGTCGC TCGGCAGCTC	1200
CTTGCTCCTA ACAGTGGAGG CCAGACTTAG GCACAGGACA ATGCCACCA CCACCACTGT	1260
GCCGCACAAG GCCGTGGCGG TAGGGTATGT GTCTGAAAT GAGCTCGGAG ATTGGGCTCG	1320
CACCGCTGAC GCAGATGGAA GACTTAAGGC AGCGGCAGAA GAAGATGCAG GCAGCTGAGT	1380
TGTTGTATTG TGTAAGTTG GAGGTAACCT CCGTTGCGGT GCTGTTAACG GTGGAGGGCA	1440
GTGTAGTCTG AGCAGTACTC GTTGCTGCCG CGCGCGCCAC CAGACATAAT AGCTGACAGA	1500
CTAACAGACT GTTCCTTTCC ATGGGTCTTT TCTGCAGTCA CCGTCCTTGA CACG ATG	1557
	Met
	1
GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA GCA	1605
Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala	
5 10 15	
GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA GGC	1653
Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg Gly	
20 25 30	
GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT GTG	1701
Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val	
35 40 45	
TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA GCA	1749

Trp	Lys	Glu	Ala	Thr	Thr	Thr	Leu	Phe	Cys	Ala	Ser	Asp	Ala	Lys	Ala	
50					55					60					65	
TAT	GAT	ACA	GAG	GTA	CAT	AAT	GTT	TGG	GCC	ACA	CAT	GCC	TGT	GTA	CCC	1797
Tyr	Asp	Thr	Glu	Val	His	Asn	Val	Trp	Ala	Thr	His	Ala	Cys	Val	Pro	
			70					75						80		
ACA	GAC	CCC	AAC	CCA	CAA	GAA	GTA	GTA	TTG	GTA	AAT	GTG	ACA	GAA	AAT	1845
Thr	Asp	Pro	Asn	Pro	Gln	Glu	Val	Val	Leu	Val	Asn	Val	Thr	Glu	Asn	
			85				90						95			
TTT	AAC	ATG	TGG	AAA	AAT	GAC	ATG	GTA	GAA	CAG	ATG	CAT	GAG	GAT	ATA	1893
Phe	Asn	Met	Trp	Lys	Asn	Asp	Met	Val	Glu	Gln	Met	His	Glu	Asp	Ile	
	100					105						110				
ATC	AGT	TTA	TGG	GAT	CAA	AGC	CTA	AAG	CCA	TGT	GTA	AAA	TTA	ACC	CCA	1941
Ile	Ser	Leu	Trp	Asp	Gln	Ser	Leu	Lys	Pro	Cys	Val	Lys	Leu	Thr	Pro	
	115					120					125					
CTC	TGT	GTT	AGT	TTA	AAG	TGC	ACT	GAT	TTG	GGG	AAT	GCT	ACT	AAT	ACC	1989
Leu	Cys	Val	Ser	Leu	Lys	Cys	Thr	Asp	Leu	Gly	Asn	Ala	Thr	Asn	Thr	
130				135					140					145		
AAT	AGT	AGT	AAT	ACC	AAT	AGT	AGT	AGC	GGG	GAA	ATG	ATG	ATG	GAG	AAA	2037
Asn	Ser	Ser	Asn	Thr	Asn	Ser	Ser	Ser	Gly	Glu	Met	Met	Met	Glu	Lys	
			150						155					160		
GGA	GAG	ATA	AAA	AAC	TGC	TCT	TTC	AAT	ATC	AGC	ACA	AGC	ATA	AGA	GGT	2085
Gly	Glu	Ile	Lys	Asn	Cys	Ser	Phe	Asn	Ile	Ser	Thr	Ser	Ile	Arg	Gly	
		165					170						175			
AAG	GTG	CAG	AAA	GAA	TAT	GCA	TTT	TTT	TAT	AAA	CTT	GAT	ATA	ATA	CCA	2133
Lys	Val	Gln	Lys	Glu	Tyr	Ala	Phe	Phe	Tyr	Lys	Leu	Asp	Ile	Ile	Pro	
	180					185						190				
ATA	GAT	AAT	GAT	ACT	ACC	AGC	TAT	ACG	TTG	ACA	AGT	TGT	AAC	ACC	TCA	2181
Ile	Asp	Asn	Asp	Thr	Thr	Ser	Tyr	Thr	Leu	Thr	Ser	Cys	Asn	Thr	Ser	
	195					200					205					
GTC	ATT	ACA	CAG	GCC	TGT	CCA	AAG	GTA	TCC	TTT	GAG	CCA	ATT	CCC	ATA	2229
Val	Ile	Thr	Gln	Ala	Cys	Pro	Lys	Val	Ser	Phe	Glu	Pro	Ile	Pro	Ile	
210				215					220					225		
CAT	TAT	TGT	GCC	CCG	GCT	GGT	TTT	GCG	ATT	CTA	AAA	TGT	AAT	AAT	AAG	2277
His	Tyr	Cys	Ala	Pro	Ala	Gly	Phe	Ala	Ile	Leu	Lys	Cys	Asn	Asn	Lys	
			230				235							240		
ACG	TTC	AAT	GGA	ACA	GGA	CCA	TGT	ACA	AAT	GTC	AGC	ACA	GTA	CAA	TGT	2325
Thr	Phe	Asn	Gly	Thr	Gly	Pro	Cys	Thr	Asn	Val	Ser	Thr	Val	Gln	Cys	
		245					250						255			
ACA	CAT	GGA	ATT	AGG	CCA	GTA	GTA	TCA	ACT	CAA	CTG	CTG	TTG	AAT	GGC	2373
Thr	His	Gly	Ile	Arg	Pro	Val	Val	Ser	Thr	Gln	Leu	Leu	Leu	Asn	Gly	
		260				265							270			
AGT	CTA	GCA	GAA	GAA	GAG	GTA	GTA	ATT	AGA	TCT	GCC	AAT	TTC	ACA	GAC	2421
Ser	Leu	Ala	Glu	Glu	Glu	Val	Val	Ile	Arg	Ser	Ala	Asn	Phe	Thr	Asp	
	275					280					285					
AAT	GCT	AAA	ACC	ATA	ATA	GTA	CAG	CTG	AAC	CAA	TCT	GTA	GAA	ATT	AAT	2469
Asn	Ala	Lys	Thr	Ile	Ile	Val	Gln	Leu	Asn	Gln	Ser	Val	Glu	Ile	Asn	
290				295					300					305		
TGT	ACA	AGA	CCC	AAC	AAC	AAT	ACA	AGA	AAA	AGT	ATC	CGT	ATC	CAG	AGG	2517
Cys	Thr	Arg	Pro	Asn	Asn	Asn	Thr	Arg	Lys	Ser	Ile	Arg	Ile	Gln	Arg	
			310				315						320			
GGA	CCA	GGG	AGA	GCA	TTT	GTT	ACA	ATA	GGA	AAA	ATA	GGA	AAT	ATG	AGA	2565
Gly	Pro	Gly	Arg	Ala	Phe	Val	Thr	Ile	Gly	Lys	Ile	Gly	Asn	Met	Arg	

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325	330	335	
CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT GCC ACT TTA AAA Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu Lys 340 345 350			2613
CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT AAT AAA ACA ATA Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr Ile 355 360 365			2661
ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT GTA ACG CAC AGT Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His Ser 370 375 380 385			2709
TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe 390 395 400			2757
AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA GGG TCA AAT AAC Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn Asn 405 410 415			2805
ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA ATA AAA CAA TTT Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Phe 420 425 430			2853
ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT CCC ATC Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile 435 440 445			2901
AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG CTG CTA TTA ACA Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr 450 455 460 465			2949
AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC TTC AGA CCT GGA Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro Gly 470 475 480			2997
GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys 485 490 495			3045
GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG AGA Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg 500 505 510			3093
AGA GTG GTG CAG AGA GAA AAA T GAGCGGCCGC Arg Val Val Gln Arg Glu Lys 515 520			3125

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1 5 10 15
 Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
 20 25 30
 Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro

60

35	40	45	
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys			
50	55	60	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val			
65	70	75	80
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu			
	85	90	95
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp			
	100	105	110
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr			
	115	120	125
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn			
	130	135	140
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu			
145	150	155	160
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg			
	165	170	175
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile			
	180	185	190
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr			
	195	200	205
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro			
	210	215	220
Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn			
225	230	235	240
Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln			
	245	250	255
Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn			
	260	265	270
Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr			
	275	280	285
Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile			
	290	295	300
Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln			
305	310	315	320
Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met			
	325	330	335
Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu			
	340	345	350
Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr			
	355	360	365
Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His			
	370	375	380
Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu			
385	390	395	400
Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn			

61

405 410 415
 Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
 420 425 430
 Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
 435 440 445
 Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu
 450 455 460
 Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro
 465 470 475 480
 Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr
 485 490 495
 Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys
 500 505 510
 Arg Arg Val Val Gln Arg Glu Lys
 515 520

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1532 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1522
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA	192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA	240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA	288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu	
85 90 95	
CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT	336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp	
100 105 110	

62

ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 115 120 125	384
CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr 130 135 140	432
ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys 145 150 155 160	480
TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr 165 170 175	528
GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr 180 185 190	576
AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys 195 200 205	624
CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala 210 215 220	672
GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly 225 230 235 240	720
CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro 245 250 255	768
GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu 260 265 270	816
GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile 275 280 285	864
GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn 290 295 300	912
AAT ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACT Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr 305 310 315 320	960
ACA GGA GAA ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser 325 330 335	1008
AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT ATA AAA TTA AGA Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg 340 345 350	1056
GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC TCC TCA GGA GGG Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly 355 360 365	1104
GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGA GAA TTT TTC Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe 370 375 380	1152
TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG AAT AAT AAT ACT	1200

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Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr	
385 390 395 400	
GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA CTC CCA TGC AGA	1248
Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg	
405 410 415	
ATA AAA CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT	1296
Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr	
420 425 430	
GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG	1344
Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly	
435 440 445	
CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT GGG ACC GAG ATC	1392
Leu Leu Leu Thr Arg Asp Gly Ile Asn Glu Asn Gly Thr Glu Ile	
450 455 460	
TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA	1440
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu	
465 470 475 480	
TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC	1488
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr	
485 490 495	
AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T GAGCGGCCGC	1532
Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys	
500 505	

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 507 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu	
85 90 95	
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp	
100 105 110	
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
115 120 125	
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr	
130 135 140	

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Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
 145 150 155 160
 Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
 165 170 175
 Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
 180 185 190
 Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
 195 200 205
 Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
 210 215 220
 Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
 225 230 235 240
 Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
 245 250 255
 Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
 260 265 270
 Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
 275 280 285
 Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
 290 295 300
 Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
 305 310 315 320
 Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
 325 330 335
 Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
 340 345 350
 Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
 355 360 365
 Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
 370 375 380
 Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
 385 390 395 400
 Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
 405 410 415
 Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
 420 425 430
 Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
 435 440 445
 Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
 450 455 460
 Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
 465 470 475 480
 Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
 485 490 495
 Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
 500 505

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1484 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1474
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA	192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA	240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA	288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu	
85 90 95	
AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT	336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp	
100 105 110	
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC	384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
115 120 125	
CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT	432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn	
130 135 140	
ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG	480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu	
145 150 155 160	
AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA	528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg	
165 170 175	
GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA	576
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile	
180 185 190	
CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC	624
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr	
195 200 205	

66

TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro 210 215 220	672
ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn 225 230 235 240	720
AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln 245 250 255	768
TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Asn 260 265 270	816
GGC AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr 275 280 285	864
GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile 290 295 300	912
AAT TGT ACA GGT GCT GGA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn 305 310 315 320	960
GCC ACT TTA AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn 325 330 335	1008
AAT AAA ACA ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile 340 345 350	1056
GTA ACG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser 355 360 365	1104
ACA CAA CTG TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu 370 375 380	1152
GGG TCA AAT AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg 385 390 395 400	1200
ATA AAA CAA TTT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr 405 410 415	1248
GCC CCT CCC ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly 420 425 430	1296
CTG CTA TTA ACA AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile 435 440 445	1344
TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu 450 455 460	1392
TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr 465 470 475 480	1440
AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA T GAGCGGCCGC	1484

SUBSTITUTE SHEET (RULE 26)

Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
485 490

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly
1 5 10 15
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
35 40 45
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
50 55 60
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
65 70 75 80
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
85 90 95
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
100 105 110
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
115 120 125
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
130 135 140
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
145 150 155 160
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
165 170 175
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
180 185 190
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
195 200 205
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
210 215 220
Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
225 230 235 240
Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
245 250 255
Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn
260 265 270
Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
275 280 285

68

Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
 290 295 300
 Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
 305 310 315 320
 Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
 325 330 335
 Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
 340 345 350
 Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
 355 360 365
 Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
 370 375 380
 Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
 385 390 395 400
 Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
 405 410 415
 Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
 420 425 430
 Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
 435 440 445
 Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
 450 455 460
 Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
 465 470 475 480
 Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
 485 490

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1448 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1439
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT STG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	

GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys 50 55 60	192
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val 65 70 75 80	240
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu 85 90 95	288
CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp 100 105 110	336
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 115 120 125	384
CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr 130 135 140	432
ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys 145 150 155 160	480
TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr 165 170 175	528
GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr 180 185 190	576
AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys 195 200 205	624
CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala 210 215 220	672
GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly 225 230 235 240	720
CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro 245 250 255	768
GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu 260 265 270	816
GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile 275 280 285	864
GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA GGT GCT GGA CAT Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His 290 295 300	912
TGT AAC ATT AGT AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val 305 310 315 320	960
ATA AAA TTA AGA GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC	1008

70

Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His	
325 330 335	
TCC TCA GGA GGG GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA	1056
Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly	
340 345 350	
GGA GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG	1104
Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp	
355 360 365	
AAT AAT AAT ACT GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA	1152
Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr	
370 375 380	
CTC CCA TGC AGA ATA AAA CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA	1200
Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly	
385 390 395 400	
AAA GCA ATG TAT GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA	1248
Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser	
405 410 415	
AAT ATT ACA GGG CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT	1296
Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn	
420 425 430	
GGG ACC GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG	1344
Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp	
435 440 445	
AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA	1392
Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly	
450 455 460	
GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA TG	1439
Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys	
465 470 475	
AGCGGCCGC	1448

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 479 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu	

85

90

95

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450 455 460
 Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
 465 470 475

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1484 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1454
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA	192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA	240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA	288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu	
85 90 95	
AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT	336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp	
100 105 110	
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC	384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
115 120 125	
CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT	432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn	
130 135 140	
ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG	480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu	
145 150 155 160	
AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA	528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg	
165 170 175	
GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA	576
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile	

73

180	185	190	
CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr 195 200 205			624
TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro 210 215 220			672
ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn 225 230 235 240			720
AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln 245 250 255			768
TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn 260 265 270			816
GGC-AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr 275 280 285			864
GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT Asn Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile 290 295 300			912
AAT TGT ACA GGT GCT GGA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn 305 310 315 320			960
GCC ACT TTA AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn 325 330 335			1008
AAT AAA ACA ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile 340 345 350			1056
GTA ACG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser 355 360 365			1104
ACA CAA CTG TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu 370 375 380			1152
GGG TCA AAT AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg 385 390 395 400			1200
ATA AAA CAA TTT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT Ile Lys Gln Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr 405 410 415			1248
GCC CCT CCC ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly 420 425 430			1296
CTG CTA TTA ACA AGA GAT GGT GGT AAT AAC AAC AAT GCG TCC GAG ATC Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile 435 440 445			1344
TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu 450 455 460			1392

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TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC 1440
 Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
 465 470 475 480

AAG GCA AAG AGA AG AGTGGTGCAG AGAGAAAAAT GAGCGGCCGC 1484
 Lys Ala Lys Arg

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 484 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1 5 10 15
 Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
 20 25 30
 Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 35 40 45
 Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
 50 55 60
 Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65 70 75 80
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu
 85 90 95
 Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp
 100 105 110
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 115 120 125
 Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn
 130 135 140
 Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu
 145 150 155 160
 Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg
 165 170 175
 Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile
 180 185 190
 Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr
 195 200 205
 Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro
 210 215 220
 Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn
 225 230 235 240
 Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln
 245 250 255
 Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn

75

260 265 270
 Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr
 275 280 285
 Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile
 290 295 300
 Asn Cys Thr Gly Ala Gly His Cys Asn Ile Ser Arg Ala Lys Trp Asn
 305 310 315 320
 Ala Thr Leu Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn
 325 330 335
 Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile
 340 345 350
 Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser
 355 360 365
 Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu
 370 375 380
 Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg
 385 390 395 400
 Ile Lys Gln Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
 405 410 415
 Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
 420 425 430
 Leu Leu Leu Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile
 435 440 445
 Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
 450 455 460
 Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
 465 470 475 480
 Lys Ala Lys Arg

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1448 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1438
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	

76

GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro 35 40 45	144
GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys 50 55 60	192
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val 65 70 75 80	240
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu 85 90 95	288
CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp 100 105 110	336
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 115 120 125	384
CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr 130 135 140	432
ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys 145 150 155 160	480
TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr 165 170 175	528
GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr 180 185 190	576
AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys 195 200 205	624
CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala 210 215 220	672
GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly 225 230 235 240	720
CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro 245 250 255	768
GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu 260 265 270	816
GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile 275 280 285	864
GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA GGT GCT GGA CAT Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His 290 295 300	912
TGT AAC ATT AGT AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT	960

SUBSTITUTE SHEET (RULE 26)

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Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val 305 310 315 320	
ATA AAA TTA AGA GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His 325 330 335	1008
TGC TCA GGA GGG GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly 340 345 350	1056
GGA GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp 355 360 365	1104
AAT AAT AAT ACT GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr 370 375 380	1152
CTC CCA TGC AGA ATA AAA CAA ATT ATA AAC ATG GTG CAG GAA GTA GGA Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly 385 390 395 400	1200
AAA GCA ATG TAT GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser 405 410 415	1248
AAT ATT ACA GGG CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn 420 425 430	1296
GGG ACC GAG ATC TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp 435 440 445	1344
AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly 450 455 460	1392
GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys 465 470 475	1438
GAGCGGCCGC	1448

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 479 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg 20 25 30
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro 35 40 45
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys 50 55 60

78

Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
 65 70 75 80
 Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
 85 90 95
 His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
 100 105 110
 Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
 115 120 125
 Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
 130 135 140
 Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
 145 150 155 160
 Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
 165 170 175
 Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
 180 185 190
 Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
 195 200 205
 Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
 210 215 220
 Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
 225 230 235 240
 Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
 245 250 255
 Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
 260 265 270
 Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
 275 280 285
 Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Gly Ala Gly His
 290 295 300
 Cys Asn Ile Ser Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val
 305 310 315 320
 Ile Lys Leu Arg Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His
 325 330 335
 Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly
 340 345 350
 Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp
 355 360 365
 Asn Asn Asn Thr Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr
 370 375 380
 Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly
 385 390 395 400
 Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser
 405 410 415
 Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn
 420 425 430

Gly Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp
435 440 445

Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly
450 455 460

Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
465 470 475

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1571 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1567
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GCC AGA ACA GAA AAA TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
GTG TGG AAG GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA	192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA	240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GTA AAT GTG ACA GAA	288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu	
85 90 95	
AAT TTT AAC ATG TGG AAA AAT GAC ATG GTA GAA CAG ATG CAT GAG GAT	336
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp	
100 105 110	
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC	384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
115 120 125	
CCA CTC TGT GTT AGT TTA AAG TGC ACT GAT TTG GGG AAT GCT ACT AAT	432
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn	
130 135 140	
ACC AAT AGT AGT AAT ACC AAT AGT AGT AGC GGG GAA ATG ATG ATG GAG	480
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu	
145 150 155 160	
AAA GGA GAG ATA AAA AAC TGC TCT TTC AAT ATC AGC ACA AGC ATA AGA	528
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg	

80

165	170	175	
GGT AAG GTG CAG AAA GAA TAT GCA TTT TTT TAT AAA CTT GAT ATA ATA Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile 180 185 190			576
CCA ATA GAT AAT GAT ACT ACC AGC TAT ACG TTG ACA AGT TGT AAC ACC Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr 195 200 205			624
TCA GTC ATT ACA CAG GCC TGT CCA AAG GTA TCC TTT GAG CCA ATT CCC Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro 210 215 220			672
ATA CAT TAT TGT GCC CCG GCT GGT TTT GCG ATT CTA AAA TGT AAT AAT Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn 225 230 235 240			720
AAG ACG TTC AAT GGA ACA GGA CCA TGT ACA AAT GTC AGC ACA GTA CAA Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln 245 250 255			768
TGT ACA CAT GGA ATT AGG CCA GTA GTA TCA ACT CAA CTG CTG TTG AAT Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn 260 265 270			816
GGC AGT CTA GCA GAA GAA GAG GTA GTA ATT AGA TCT GCC AAT TTC ACA Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr 275 280 285			864
GAC AAT GCT AAA ACC ATA ATA GTA CAG CTG AAC CAA TCT GTA GAA ATT Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile 290 295 300			912
AAT TGT ACA AGA CCC AAC AAC AAT ACA AGA AAA AGT ATC CGT ATC CAG Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln 305 310 315 320			960
AGG GGA CCA GGG AGA GCA TTT GTT ACA ATA GGA AAA ATA GGA AAT ATG Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met 325 330 335			1008
AGA CAA GCA CAT TGT AAC ATT AGT AGA GCA AAA TGG AAT GCC ACT TTA Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu 340 345 350			1056
AAA CAG ATA GCT AGC AAA TTA AGA GAA CAA TTT GGA AAT AAT AAA ACA Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr 355 360 365			1104
ATA ATC TTT AAG CAA TCC TCA GGA GGG GAC CCA GAA ATT GTA ACG CAC Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His 370 375 380			1152
AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC TGT AAT TCA ACA CAA CTG Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu 385 390 395 400			1200
TTT AAT AGT ACT TGG TTT AAT AGT ACT TGG AGT ACT GAA GGG TCA AAT Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn 405 410 415			1248
AAC ACT GAA GGA AGT GAC ACA ATC ACA CTC CCA TGC AGA ATA AAA CAA Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln 420 425 430			1296
TTT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT CCC Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro 435 440 445			1344

SUBSTITUTE SHEET (RULE 26)

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ATC AGC GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG CTG CTA TTA Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu 450 455 460	1392
ACA AGA GAT GGT GGT AAT AAC AAC AAT GGG TCC GAG ATC TTC AGA CCT Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro 465 470 475 480	1440
GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr 485 490 495	1488
AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys 500 505 510	1536
AGA AGA GTG GTG CAG AGA GAA AAA TGA GCG G CCGC Arg Arg Val Val Gln Arg Glu Lys 515 520	1571

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 522 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg 20 25 30
Gly Ala Arg Thr Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro 35 40 45
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys 50 55 60
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val 65 70 75 80
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu 85 90 95
Asn Phe Asn Met Trp Lys Asn Asp Met Val Glu Gln Met His Glu Asp 100 105 110
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr 115 120 125
Pro Leu Cys Val Ser Leu Lys Cys Thr Asp Leu Gly Asn Ala Thr Asn 130 135 140
Thr Asn Ser Ser Asn Thr Asn Ser Ser Ser Gly Glu Met Met Met Glu 145 150 155 160
Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg 165 170 175
Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile 180 185 190
Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr

82

195	200	205
Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro		
210	215	220
Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asn		
225	230	235 240
Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn Val Ser Thr Val Gln		
	245	250 255
Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn		
	260	265 270
Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe Thr		
	275	280 285
Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile		
	290	295 300
Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln		
	305	310 315 320
Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met		
	325	330 335
Arg Gln Ala His Cys Asn Ile Ser Arg Ala Lys Trp Asn Ala Thr Leu		
	340	345 350
Lys Gln Ile Ala Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr		
	355	360 365
Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His		
	370	375 380
Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu		
	385	390 395 400
Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Glu Gly Ser Asn		
	405	410 415
Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln		
	420	425 430
Phe Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro		
	435	440 445
Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu		
	450	455 460
Thr Arg Asp Gly Gly Asn Asn Asn Asn Gly Ser Glu Ile Phe Arg Pro		
	465	470 475 480
Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr		
	485	490 495
Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys		
	500	505 510
Arg Arg Val Val Gln Arg Glu Lys		
	515	520

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1532 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1522

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA	48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly	
1 5 10 15	
GCA GTC TTC GTT TCG CCC AGC CAG GAA ATC CAT GCC CGA TTC AGA AGA	96
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg	
20 25 30	
GGC GGC AGA GTA GAA AAG TTG TGG GTC ACA GTC TAT TAT GGG GTA CCT	144
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro	
35 40 45	
GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT AAA	192
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys	
50 55 60	
GCA TAT GAT ACA GAG GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT GTA	240
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val	
65 70 75 80	
CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GAA AAT GTA ACA GAA	288
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu	
85 90 95	
CAT TTT AAC ATG TGG AAA AAT AAC ATG GTA GAA CAG ATG CAG GAG GAT	336
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp	
100 105 110	
ATA ATC AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA ACC	384
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr	
115 120 125	
CCA CTC TGT GTT ACT TTA AAT TGC AAG GAT GTG AAT GCT ACT AAT ACC	432
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr	
130 135 140	
ACT AAT GAT AGC GAG GGA ACG ATG GAG AGA GGA GAA ATA AAA AAC TGC	480
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys	
145 150 155 160	
TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT GAG GTG CAG AAA GAA TAT	528
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr	
165 170 175	
GCT CTT TTT TAT AAA CTT GAT GTA GTA CCA ATA GAT AAT AAT AAT ACC	576
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr	
180 185 190	
AGC TAT AGG TTG ATA AGT TGT GAC ACC TCA GTC ATT ACA CAG GCC TGT	624
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys	
195 200 205	
CCA AAG ATA TCC TTT GAG CCA ATT CCC ATA CAT TAT TGT GCC CCG GCT	672
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala	
210 215 220	
GGT TTT GCG ATT CTA AAG TGT AAT GAT AAG ACG TTC AAT GGA AAA GGA	720
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly	
225 230 235 240	

84

CCA TGT AAA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro 245 250 255	768
GTA GTA TCA ACT CAA CTG CTG CTA AAT GGC AGT CTA GCA GAA GAA GAG Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu 260 265 270	816
GTA GTA ATT AGA TCT GAC AAT TTC ACG AAC AAT GCT AAA ACC ATA ATA Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile 275 280 285	864
GTA CAG CTG AAA GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn 290 295 300	912
AAT ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACT Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr 305 310 315 320	960
ACA GGA GAA ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser 325 330 335	1008
AGA GCA AAA TGG AAT GAC ACT TTA AAA CAG ATA GTT ATA AAA TTA AGA Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg 340 345 350	1056
GAA CAA TTT GAG AAT AAA ACA ATA GTC TTT AAT CAC TCC TCA GGA GGG Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly 355 360 365	1104
GAC CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGA GAA TTT TTC Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe 370 375 380	1152
TAC TGT AAT TCA ACA CAA CTG TTT AAT AGT ACT TGG AAT AAT AAT ACT Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr 385 390 395 400	1200
GAA GGG TCA AAT AAC ACT GAA GGA AAT ACT ATC ACA CTC CCA TGC AGA Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg 405 410 415	1248
ATA AAA CAA ATT ATA AAC ATG GTG CAG GAA GTA GGA AAA GCA ATG TAT Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr 420 425 430	1296
GCC CCT CCC ATC AGA GGA CAA ATT AGA TGT TCA TCA AAT ATT ACA GGG Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly 435 440 445	1344
CTG CTA TTA ACA AGA GAT GGT GGT ATT AAT GAG AAT GGG ACC GAG ATC Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile 450 455 460	1392
TTC AGA CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu 465 470 475 480	1440
TAT AAA TAT AAA GTA GTA AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr 485 490 495	1488
AAG GCA AAG AGA AGA GTG GTG CAA AGA GAA AAA T GAGCGGCGCG Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys 500 505	1532

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 507 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1           5           10           15
Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
      20           25           30
Gly Gly Arg Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
      35           40           45
Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
      50           55           60
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
      65           70           75           80
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Glu Asn Val Thr Glu
      85           90           95
His Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met Gln Glu Asp
      100          105          110
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
      115          120          125
Pro Leu Cys Val Thr Leu Asn Cys Lys Asp Val Asn Ala Thr Asn Thr
      130          135          140
Thr Asn Asp Ser Glu Gly Thr Met Glu Arg Gly Glu Ile Lys Asn Cys
      145          150          155          160
Ser Phe Asn Ile Thr Thr Ser Ile Arg Asp Glu Val Gln Lys Glu Tyr
      165          170          175
Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asn Asn Thr
      180          185          190
Ser Tyr Arg Leu Ile Ser Cys Asp Thr Ser Val Ile Thr Gln Ala Cys
      195          200          205
Pro Lys Ile Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala
      210          215          220
Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Thr Phe Asn Gly Lys Gly
      225          230          235          240
Pro Cys Lys Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro
      245          250          255
Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu
      260          265          270
Val Val Ile Arg Ser Asp Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile
      275          280          285
Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn
      290          295          300
Asn Thr Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr

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305              310              315              320
Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser
      325              330              335
Arg Ala Lys Trp Asn Asp Thr Leu Lys Gln Ile Val Ile Lys Leu Arg
      340              345              350
Glu Gln Phe Glu Asn Lys Thr Ile Val Phe Asn His Ser Ser Gly Gly
      355              360              365
Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe
      370              375              380
Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Asn Thr
      385              390              395              400
Glu Gly Ser Asn Asn Thr Glu Gly Asn Thr Ile Thr Leu Pro Cys Arg
      405              410              415
Ile Lys Gln Ile Ile Asn Met Val Gln Glu Val Gly Lys Ala Met Tyr
      420              425              430
Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly
      435              440              445
Leu Leu Leu Thr Arg Asp Gly Gly Ile Asn Glu Asn Gly Thr Glu Ile
      450              455              460
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
      465              470              475              480
Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr
      485              490              495
Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys
      500              505

```

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

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Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
1           5           10           15

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What is claimed is:

1. A recombinant nucleic acid molecule which encodes a mutant HIV-1 gp120 envelope glycoprotein comprising a V3 loop deletion and a C4 domain_(W->X) point mutation, wherein X is an amino acid residue other than tryptophan.
2. The recombinant nucleic acid molecule of claim 1, wherein X is a valine residue.
3. The recombinant nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.
4. The recombinant nucleic acid molecule of claim 3, wherein the DNA molecule is a plasmid.
5. The recombinant nucleic acid molecule of claim 4, wherein the plasmid comprises the sequence of the plasmid designated PPI4-tPA.
6. The recombinant nucleic acid molecule of claim 1, wherein the C4 domain is an HIV-1_{LA1} gp120 envelope glycoprotein C4 domain.
7. The recombinant nucleic acid molecule of claim 6, wherein the mutant HIV-1 gp120 envelope glycoprotein is a mutant HIV-1_{LA1} gp120 envelope glycoprotein.
8. The recombinant nucleic acid molecule of claim 1, wherein the C4 domain is an HIV-1_{RF1} gp120 envelope glycoprotein C4 domain.
9. The recombinant nucleic acid molecule of claim 8,

wherein the mutant HIV-1 gp120 envelope glycoprotein is a mutant HIV-1_{JR-FL} gp120 envelope glycoprotein.

10. The mutant HIV-1 gp120 envelope glycoprotein encoded by the recombinant nucleic acid molecule of claim 1.
11. A vaccine which comprises a therapeutically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of claim 10, and an adjuvant.
12. A method of treating an HIV-1-infected subject, which comprises immunizing the HIV-1-infected subject with the vaccine of claim 11, thereby treating the HIV-1-infected subject.
13. A vaccine which comprises a prophylactically effective amount of the mutant HIV-1 gp120 envelope glycoprotein of claim 10, and an adjuvant.
14. A method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the HIV-1-exposed subject with the vaccine of claim 13, thereby reducing the likelihood of the HIV-1-exposed subject's becoming infected with HIV-1.
15. A method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1, which comprises immunizing the non-HIV-1-exposed subject with the vaccine of claim 13, thereby reducing the likelihood of the non-HIV-1-exposed subject's becoming infected with HIV-1.
16. A method of obtaining partially purified antibodies which specifically bind to the CD4-binding domain of

HIV-1 gp120 envelope glycoprotein, which method comprises (a) immunizing a non-HIV-1-exposed subject with the vaccine of claim 13, (b) recovering from the immunized subject serum comprising said antibodies, and
5 (c) partially purifying said antibodies, thereby obtaining partially purified antibodies which specifically bind to the CD4-binding domain of HIV-1 gp120 envelope glycoprotein.

10 17. The method of claim 16, wherein the subject is a human.

18. The partially purified antibodies produced by the method of claim 16.

15 19. A pharmaceutical composition, which comprises a therapeutically effective amount of the partially purified antibodies of claim 18, and a pharmaceutically acceptable carrier.

20 20. A method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of claim 19 effective to reduce the population of HIV-1-infected cells in the HIV-1-infected subject, thereby treating the HIV-1-
25 infected subject.

21. A method of treating an HIV-1-infected subject, which comprises administering to the subject a dose of the pharmaceutical composition of claim 19 effective to
30 reduce the population of HIV-1 in the HIV-1-infected subject, thereby treating the HIV-1-infected subject.

22. A composition which comprises a prophylactically effective amount of the partially purified antibodies
35 of claim 18, and a pharmaceutically acceptable carrier.

23. A method of reducing the likelihood of an HIV-1-exposed subject's becoming infected with HIV-1, which comprises administering to the HIV-1-exposed subject a dose of the composition of claim 22 effective to reduce the population of HIV-1 in the HIV-1-exposed subject, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

24. The method of claim 23, wherein the subject is a medical practitioner.

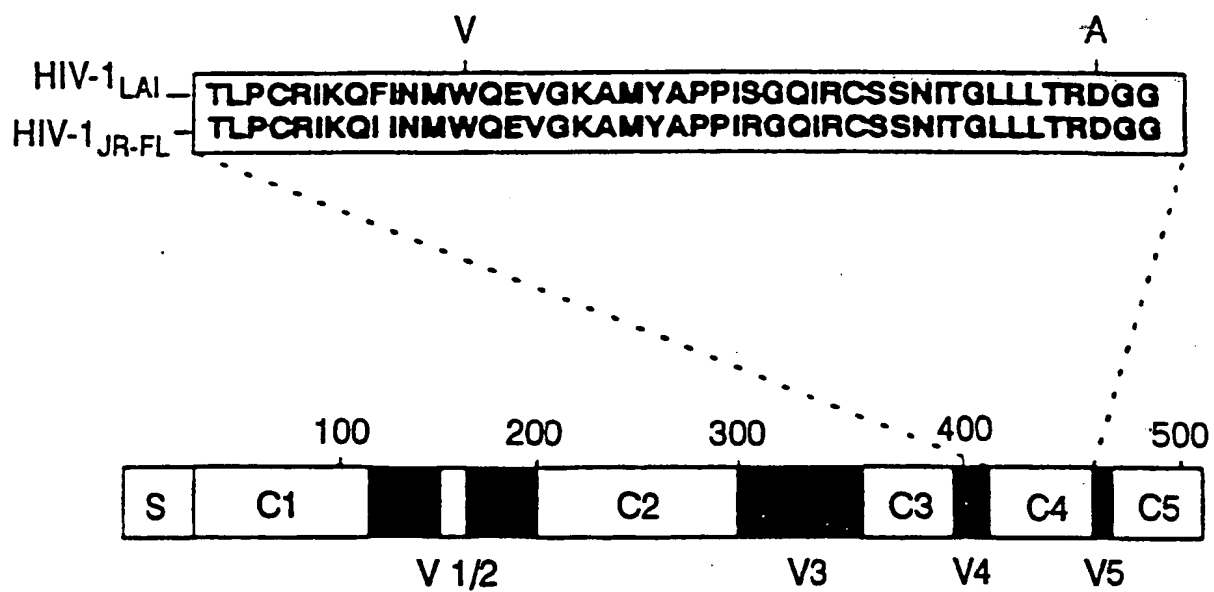
25. The method of claim 23, wherein the subject is a newborn infant.

26. A method of reducing the likelihood of a non-HIV-1-exposed subject's becoming infected with HIV-1 as a result of exposure thereto during an incident wherein there is an increased risk of exposure to HIV-1, which comprises administering to the subject immediately prior to the incident a dose of the composition of claim 22 effective to reduce the population of HIV-1 to which the subject is exposed during the incident, thereby reducing the likelihood of the subject's becoming infected with HIV-1.

27. The method of claim 26, wherein the subject is a medical practitioner.

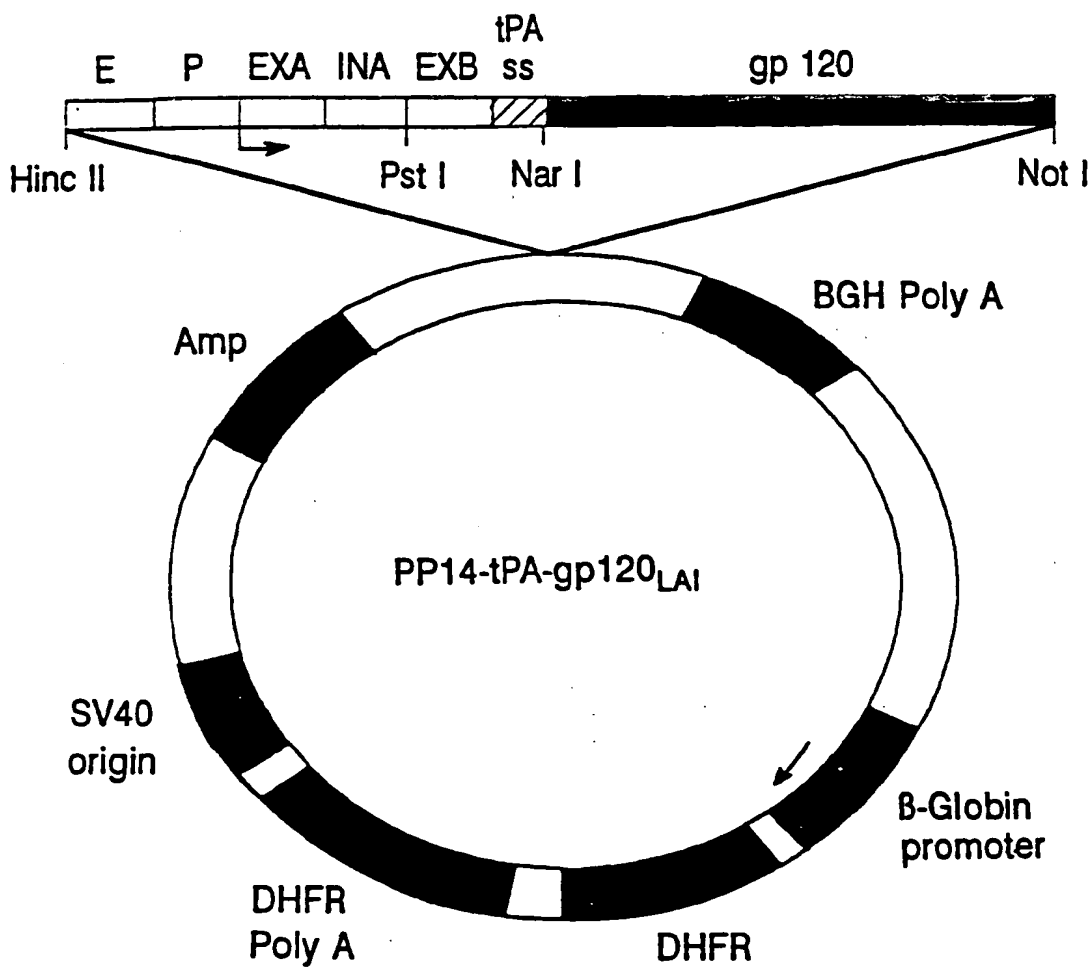
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FIGURE 1



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FIGURE 2



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FIGURE 3A
FIGURE 3B
FIGURE 3C
FIGURE 3D
FIGURE 3E
FIGURE 3F

FIGURE 3A

HincII

1 ttgacattgattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatgga
 73 gttccgcgttacataacttacggtaaatggccgcctggctgacggcccaacgacccccgccattgacgtc
 145 aataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggactatttacg
 217 gtactgcccacttggcagtagacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaatgacgg
 289 taaatggccgcctggcattatgcccagtagaccttatgggactttcctacttggcagtacatctacgt
 361 attagtcacgcctattaccatggtgatgcggttttggcagtagacatcaatgggcgtggatagcgggttggactc
 433 acggggatttccaagtctccaccccatgacgtcaatgggagtttgttttggcaccacaaatcaacggggactt

FIGURE 3B

505 tccaaaatgtcgtaaacaactccgcccattgacgcgaatgggcggtaggcgtgtacgggtgggaggtctatat
Exon A
577 aagcagagctcgtttaagtgaaccgtcagatcgccctggagacgccatccacgctgttttgacctccatagaag
→ Transcription Start
649 ACACGGGACCGATCCAGCCTCCGGCGCGGAACGGTGCAATTGGAACGCCGATTCCCCGTGCCAAGAGTGA
Intron A
721 Cgtaagtaccgcctatagactctataggcacaccccctttggctcttatgcatgctatactgtttttggcttg
793 ggccaacaccccgtcctagataggtgatggtatagcttagcctatagggtgtgggttattgaccattattgac
865 cactccccctattggtgacgatactttccattactaatccataaacatggccgctctttgccacaactatctct
937 attggctatatgccaatactctgtccttcagagactgacaaggactctgtatttttacaggatgggggtccca
11009 ttattattacaaaattcacatatatacaaacgcgctccccgtgcccgcagtttttattaacatgcgggat
11081 ctccacgcgaatctcgggtacgtgttcgggacatgggctcttctccggtagcggcgagctccacatccgag
11153 cctgtcccatgcccatgcctccagcggctcatggtcgctcggcagctccttgctcctaacagtggaggccag
1225 acttaggcacaggacaatgccaccaccacaggtgtgccgcacaaggccgtggcggtaggggtatgtgtctga

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FIGURE 3D

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1873 GAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAGCCCTAAAGCCATGTGTAAATTAACCCCACTC
107  E Q M H E D I I S L W D Q S L K P C V K L T P L

1945 TGTGTTAGTTTAAAGTGCACTGATTGGGGAATGCTACTAATACCAATAGTAGTAATACCAATAGTAGTAGC
131  C V S L K C T D L G N A T N T N S S N T N S S S

2017 GGGGAAATGATGATGGAGAAAGGAGAGATAAAACTGCTCTTTCATATCAGCACAAAGCATAAGAGGTAAG
155  G E M M E K G E I K N C S F N I S T S I R G K

2089 GTGCAGAAAGAATATGCAATTTTTTATAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
179  V Q K E Y A F F Y K L D I I P I D N D T T S Y T

2161 TTGACAAGTTGTAACACCTCAGTCATTACACAGGCGCTGCCAAGGTATCCTTTGAGCCCAATTCCCATACAT
203  L T S C N T S V I T Q A C P K V S F E P I P I H

2233 TATTGTGCCCCGGCTGTTTTCGGATTCTAAATGTAATAAAGACGTTCAATGGAACAGGACCATGTACA
227  Y C A P A G F A I L K C N N K T F N G T G P C T

2305 AATGTCAGCACAGTACATGTACACATGGAATTAGGCCAGTAGTATCAACCTCAACTGCTGTTGAATGGCAGT
251  N V S T V Q C T H G I R P V V S T Q L L L N G S

2377 CTAGCAGAGAGAGGTAGTAATTAGATCTGCCAATTTCCACAGACAATGCTAAACCATTAATAGTACAGCTG
275  L A E E E V V I R S A N F T D N A K T I I V Q L

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FIGURE 3E

2449 AACCAATCTGTAGAAATTAATTGTACAAGACCCCAACAACAATACAAGAAAGTATCCGTATCCAGAGGGGA
299 N Q S V E I N C T R P N N N T R K S I R I Q R G

2521 CCAGGAGAGCATTTGTTACAATAGGAAATATAGGAATATGAGACAAGCACATTGTAAACATTAGTAGAGCA
323 P G R A F V T I G K I G N M R Q A H C N I S R A

2593 AAATGGAATGCCACTTTTAAACAGATAGCTAGCAAAATTAAGAGAACAATTTGGAATAATAAACAATAATC
347 K W N A T L K Q I A S K L R E Q F G N N K T I I

2665 TTTAAGCAATCCTCAGGAGGGACCCAGAAATTGTAAACGCACAGTTTAAATTGTGGAGGGGAATTTTCTAC
371 F K Q S S G G D P E I V T H S F N C G G E F Y

2737 TGTAAATTCACACAACACTGTTAATAGTACTTGGTTAATAGTACTTGGAGTACTGAAGGGTCAAATAACACT
395 C N S T Q L F N S T W F N S T W S T E G S N N T

2809 GAAGGAAGTGACACAATCACACTCCCATGCAGAAATAAACAATTTATAAACATGTGGCAGGAAGTAGGAAA
419 E G S D T I T L P C R I K Q F I N M W Q E V G K

2881 GCAATGTATGCCCTCCCATCAGCGGACAAATTAGATGTTTCATCAAAATATTACAGGGCTGCTATTAACAAGA
443 A M Y A P P I S G Q I R C S S N I T G L L L T R

2953 GATGGTGTAAACAACAATGGGTCGAGATCTTCAGACCCTGGAGGAGGAGATATGAGGACAATTGGAGA
467 D G G N N N G S E I F R P G G G D M R D N W R

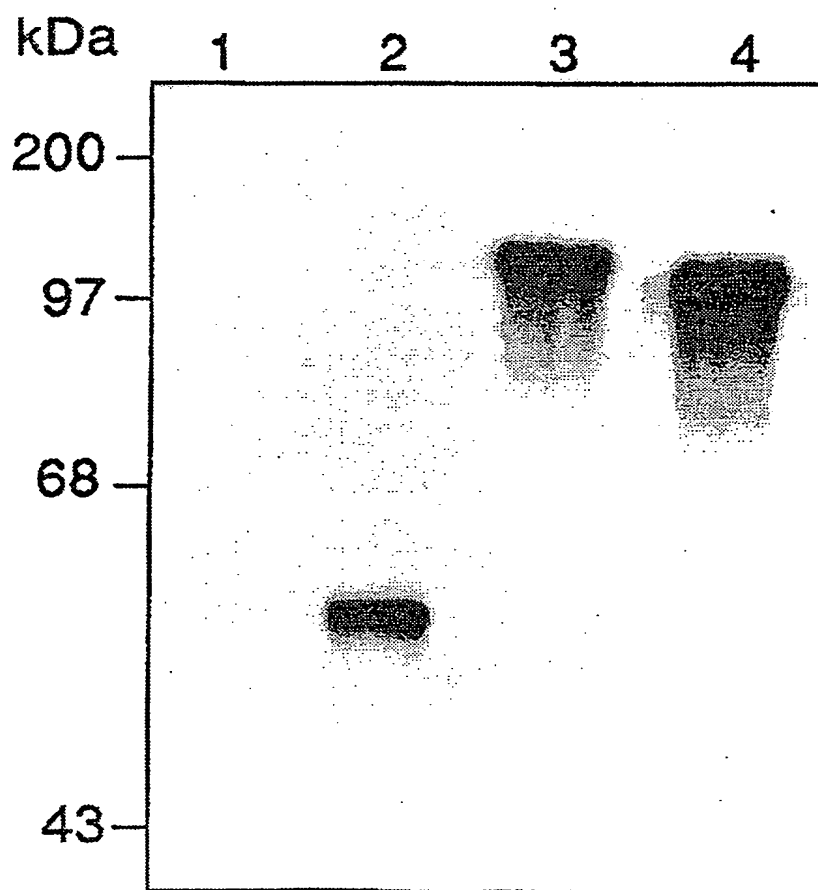
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FIGURE 3F

3025	AGTGAATTATATATAAAGTAGTAAATAATTGAACCATTTAGGAGTAGCACCCACCAAGGCAAAGAGAAGA
491	S E L Y K Y K V V K V I E P L G V A P T K A K R R
	NotI
3097	GTGGTGCAGAGAGAAAATGAGCGGCGCGC
515	V V Q R E K -

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FIGURE 4



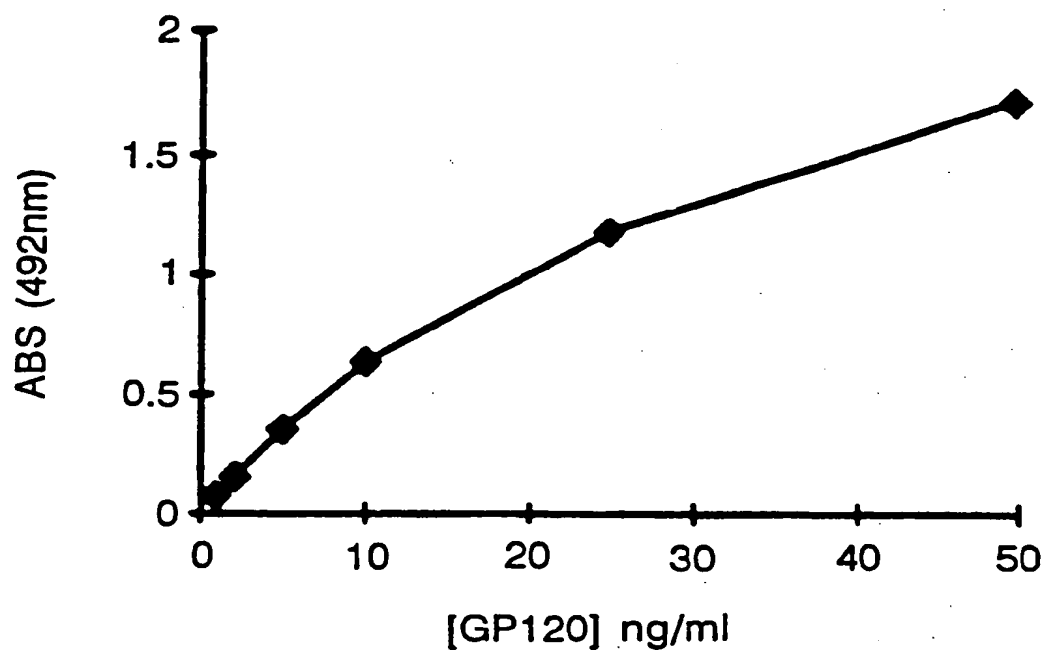
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FIGURE 5A

Stable CHO clone	[gp120] (ng/ml)
5	6
6	14
9	123
10	4
12	18
13	18

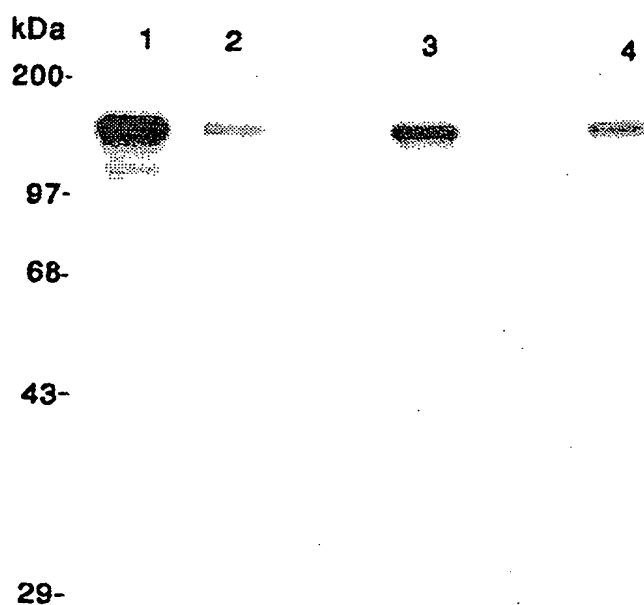
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FIGURE 5B



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FIGURE 6



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FIGURE 7A

FIGURE 7A
FIGURE 7B
FIGURE 7C

JR-FL

1 ATGGATGCAATGAAGAGA
 1 M D A M K R

 19 GGGCTCTGCTGTGCTGTGCTGTGGAGCAGTCTTCGTTTCGCCAGCCAGGAATC
 7 G L C C V L L L C G A V F V S P S Q E I
 NaI
 79 CATGCCCGATTTCAGAAAGAGCGCGCAGAGTAGAAAGTTGTGGGTCACAGTCTATTATGGG
 27 H A R F R R G A R V E K L W V T V Y Y G
 ▲ Signal cleavage
 139 GTACCTGTGTGGAAGAAGCAACCACCCTCTATTTTGTGCATCAGATGCTAAAGCATAT
 47 V P V W K E A T T T L F C A S D A K A Y

 199 GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCA
 67 D T E V H N V W A T H A C V P T D P N P

 259 CAAGAAGTAGTATTGGAAATGTAACAGAACATTTTAACATGTGGAATAAATACATGGTA
 87 Q E V V L E N V T E H F N M W K N N M V

 319 GAACAGATGCAGGAGGATATAATCAGTTTATGGATCAAAGCCTAAAGCCATGTGTAAAA
 107 E Q M Q E D I I S L W D Q S L K P C V K

 379 TTAACCCCACTCTGTGTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACCTAAT
 127 L T P L C V T L N C K D V N A T N T T N

 439 GATAGCGGGAACGATGGAGAGAGGAGAAATAAAAACTGCTCTTTCAATATCACCACA
 147 D S E G T M E R G E I K N C S F N I T T

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FIGURE 7B

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499 AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTATATAAATTGATGTAGTACCA
167 S I R D E V Q K E Y A L F Y K L D V V P

559 ATAGATAATAATAACCAGCTATAGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187 I D N N N T S Y R L I S C D T S V I T Q

619 GCCTGTCCAAGATATCCTTTGAGCCAATTCCCATACATTATTGTCCCGGCTGTTT
207 A C P K I S F E P I P I H Y C A P A G F

679 GCGATTCTAAGTGTAATGATAAGACGTTCAATGGAAAGGACCATGTAAAAATGTCAGC
227 A I L K C N D K T F N G K G P C K N V S

739 ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247 T V Q C T H G I R P V V S T Q L L L N G

799 AGTCTAGCAGAAGAGGTAGTAATTAGATCTGACAATTCACGAACAATGCTAAACC
267 S L A E E V V I R S D N F T N N A K T

859 ATAATAGTACAGCTGAAGAATCTGTAGAAATTAATTGTACAAGACCCCAACAATACA
287 I I V Q L K E S V E I N C T R P N N T

919 AGAAAAGTATACATATAGGACCAGGAGAGCATTTTATCTACAGGAGAAATAATAGGA
307 R K S I H I G P G R A F Y T T G E I I G

979 GATATAAGACACGACATTTGTAACATTAGTAGAGCAAAATGGAATGACACTTTAAACAG
327 D I R Q A H C N I S R A K W N D T L K Q

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FIGURE 7C

1039 ATAGTTATAAAATTAAGAGAACAAATTTGAGAATAAAACAATAGTCTTTAATCACTCCTCA
 347 I V I K L R E Q F E N K T I V F N H S S

 1099 GGAGGGACCCAGAAATTGTAATGCACAGTTTAAATTGGAGGAGAATTTTCTACTGT
 367 G G D P E I V M H S F N C G G E F F Y C

 1159 AATCAACACAACACTGTTTAATAGTACTTGGATAATAATACTGAAGGGTCAATAACACT
 387 N S T Q L F N S T W N N N T E G S N N T

 1219 GAAGGAAATACTATCACACTCCCATGCAGAATAAAACAATTAATAACAATGTGCAGGAA
 407 E G N T I T L P C R I K Q I I N M W Q E

 1279 GTAGGAAAGCAATGTATGCCCTCCCATCAGAGGACAAATTAGATGTTTCATCAAAATAT
 427 V G K A M Y A P P I R G Q I R C S S N I

 1339 ACAGGGCTGCTATTAAACAAGAGATGGTGTATTAAATGAGAAATGGACCGAGATCTTCAGA
 447 T G L L L T R D G G I N E N G T E I F R

 1399 CCTGGAGGAGATATGAGGACAATTTGGAGAAGTGAATTATATAAATATAAGTAGTA
 467 P G G G D M R D N W R S E L Y K Y K V V

 1459 AAAATTGAACCATTAGGAGTAGCACCCACCAGGCAAGAGAGAGTGGTGCAAGAGAA
 487 K I E P L G V A P T K A K R R V V Q R E

 1519 AAATGAGGGGGCGC
 507 K

NotI

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FIGURE 8A

FIGURE 8A
FIGURE 8B
FIGURE 8C

LAI ΔV3

1 ATGGATGCAATGAAGAGAGGGCTCTGTGTGTGCTG
 1 M D A M K R G L C C V L
 37 CTGCTGTGTGGAGCAGTCTTCGTTTCGCCAGCCAGGAATCCATGCCGATTTCAGAAGAGGCGGCCAGAACAA
 13 L L C G A V F V S P S Q E I H A R F R R G A R T
 109 GAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACCTCTATTTTGTGCA
 37 E K L W V T V Y Y G V P V W K E A T T T L F C A
 181 TCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCCTGTGTACCCACAGACCCC
 61 S D A K A Y D T E V H N V W A T H A C V P T D P
 253 AACCCACAAGTAGTATTGGTAAATGTGACAGAAATTTTAACATGTGGAAATGACATGGTAGAACAG
 85 N P Q E V V L V N V T E N F N M W K N D M V E Q
 325 ATGCATGAGGATATAATCAGTTTATGGGATCAAGCCCTAAAGCCATGTGTAAATTAACCCCACTCTGTGTT
 109 M H E D I I S L W D Q S L K P C V K L T P L C V
 397 AGTTTAAAGTGCACCTGATTGGGGAATGCTACTATAATACCAATAGTAGTAATAGTAGCGGGAA
 133 S L K C T D L G N A T N T N S S N T N S S S G E

NarI

Signal cleavage ▲

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FIGURE 8B

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469 ATGATGATGGAGAAAGGAGAGATAAAAACCTGCTCTTTTCAATATCAGCACAGCATAAGAGGTAAGGTGCAG
157 M M M E K G E I K N C S F N I S T S I R G K V Q

541 AAAGAATATGCATTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACGTTGACA
181 K E Y A F F Y K L D I I P I D N D T T S Y T L T

613 AGTTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAGCCCAATCCCATACATTATTGT
205 S C N T S V I T Q A C P K V S F E P I P I H Y C

685 GCCCCGGCTGTTTGGGATTCTAAAATGTAATAAAGACGTTCAATGGAACAGGACCATGTACAAATGTC
229 A P A G F A I L K C N N K T F N G T G P C T N V

757 AGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTGTAATGGCAGTCTAGCA
253 S T V Q C T H G I R P V V S T Q L L L N G S L A

829 GAAGAAGAGGTAGTAATTAGATCTGCCAATTTACACAGACAATGCTAAACCAATAATAGTACAGCTGAACCAA
277 E E E V V I R S A N F T D N A K T I I V Q L N Q

901 TCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAA
301 S V E I N C T G A G H C N I S R A K W N A T L K

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FIGURE 8C

973 CAGATAGCTAGCAAAATTAAGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAATCCTCAGGAGGG
 325 Q I A S K L R E Q F G N N K T I I F K Q S S G G
 1045 GACCCAGAAAATTGTAACGCACAGTTTAAATTGTGGAGGGGAATTTTCTACTGTAATTCAACACAACCTGTTT
 349 D P E I V T H S F N C G G E F F Y C N S T Q L F
 1117 AATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAATAAACAACACTGAAGGAAGTGACACAATCACA
 373 N S T W F N S T W S T E G S N N T E G S D T I T
 1189 CTCCCATGCAGAATAAAACAATTTATAACATGTGCAGGAAGTAGGAAAGCAATGTATGCCCTCCCATC
 397 L P C R I K Q F I N M W Q E V G K A M Y A P P I
 1261 AGCGGACAAATTAGATGTTTCATCAATAATTACAGGGCTGCTATTACAAGAGATGGTGGTAATAACAACAAT
 421 S G Q I R C S S N I T G L L L T R D G G N N N
 1333 GGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAAATTGGAGAAGTGAATTATATAATATAA
 445 G S E I F R P G G G D M R D N W R S E L Y K Y K
 1405 GTAGTAAAAATTGAACCATTAGGAGTAGCACCACCAAGCAAGAGAGAGTGGTGCAGAGAGAAAATGA
 469 V V K I E P L G V A P T K A K R R V V Q R E K -
 NotI
 1477 GCGGCGCG

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FIGURE 9B

439 GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAACTGCTCTTTCAATATCACCACA
147 D S E G T M E R G E I K N C S F N I T T
499 AGCATAAGAGATGAGGTGCAGAAAGAATAATGCTCTTTTATAAACTTGATGTAGTACCA
167 S I R D E V Q K E Y A L F Y K L D V V P
559 ATAGATAATAATAACCAGCTATAGGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187 I D N N N T S Y R L I S C D T S V I T Q
619 GCCTGTCCAAAGATATCCTTTGAGCCAAATTCCTCCATACATTATTGTGCCCCGGCTGTTT
207 A C P K I S F E P I P I H Y C A P A G F
679 GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAAGGACCATGTAAAAATGTCAGC
227 A I L K C N D K T F N G K G P C K N V S
739 ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247 T V Q C T H G I R P V V S T Q L L L N G
799 AGTCTAGCAGAAGAGAGGTAGTAATTAGATCTGACAATTCACGAACAATGCTAAACC
267 S L A E E E V V I R S D N F T N N A K T
859 ATAATAGTACAGCTGAAAGAATCTGTAGAAATTAATTGTACAGGCTGCGACATTGTAAAC
287 I I V Q L K E S V E I N C T G A G H C N
919 ATTAGTAGCAAAATGGAATGACACTTTAAACAGATAGTTATAAAATTAAGAGAACAA
307 I S R A K W N D T L K Q I V I K L R E Q

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FIGURE 9C

979 TTTGAGATAAAACAATAGTCTTTAATCACTCCTCAGGAGGGACCCAGAAATTGTAATG
 327 F E N K T I V F N H S S G G D P E I V M

 1039 CACAGTTTAAATTGTGGAGGAGAATTTTCTACTGTAAATTCACACAACTGTTAATAGT
 347 H S F N C G G E F F Y C N S T Q L F N S

 1099 ACTTGGAAATAATACTGAAGGGTCAATAACACTGAAGGAAATACATCACACTCCCA
 367 T W N N N T E G S N N T E G N T I T L P

 1159 TGCAGAAATAAAACAATTATAAACATGTGGCAGGAAGTAGGAAAGCAATGTATGCCCT
 387 C R I K Q I I N M W Q E V G K A M Y A P

 1219 CCCATCAGAGGACAAATTAGATGTTTCATCAATAATTACAGGGCTGCTATTAAACAAGAGT
 407 P I R G Q I R C S S N I T G L L T R D

 1279 GGTGGTATTATGAGAATGGACCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGAC
 427 G G I N E N G T E I F R P G G G D M R D

 1339 AATTGGAGAAGTGAATTATATAATAATAAGTAGTAAATAATTGAACCATTAGGAGTAGCA
 447 N W R S E L Y K Y K V V K I E P L G V A

 1399 CCCACCAAGGCAAGAGAAGAGTGGTGCAAGAGAAATGAGGGGGCC
 487 P T K A K R R V V Q R E K -

Noti

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FIGURE 10A

FIGURE 10A
FIGURE 10B
FIGURE 10C

LAI AV3-CD4-

1 ATGGATGCAATGAAGAGAGGGCTCTGTGTGTGCTG
 1 M D A M K R G L C C V L
 37 CTGCTGTGGAGCAGTCTTGGTTTCGCCCCAGCCAGGAATCCATGCCCGATTTCAGAAGAGGGCCGAGAACAA
 13 L L C G A V F V S P S Q E I H A R F R R G A R T
 109 GAAAAATTGTGGTCACAGTCTATTATGGGTACCTGTGTGGAAGGAAGCAACCACCTCTATTTGTGCA
 37 E K L W V T V Y Y G V P V W K E A T T T L F C A
 181 TCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
 61 S D A K A Y D T E V H N V N A T H A C V P T D P
 253 AACCCACAAGAGTAGTATTGGTAAATGTGACAGAAAATTTAAACATGTGGAATAATGACATGGTAGAACAG
 85 N P Q E V V L V N V T E N F N M W K N D M V E Q
 325 ATGCATGAGGATATAATCAGTTTATGGGATCAAGCCCTAAAGCCATGTGTAAATAATTAACCCACTCTGTGTT
 109 M H E D I I S L W D Q S L K P C V K L T P L G V
 397 AGTTAAAGTGCACCTGATTGGGGAATGCTACTAATAACCAATAGTAGTAATACCAATAGTAGCGGGAA
 133 S L K C T D L G N A T N T N S S N T N S S S G E

NarI

Signal cleavage ▲

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FIGURE 10B

469 ATGATGATGGAGAAAGGAGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAAGCATAAGAGGTAAGGTGCAG
157 M M E K G E I K N C S F N I S T S I R G K V Q

541 AAAGAATATGCATTTTTTATAAACTTGATATAATACCAATAGATAATGATACCTACCAGCTATACGTTGACA
181 K E Y A F F Y K L D I I P I D N D T T S Y T L T

613 AGTTGTAACACCTCAGTCATTACACAGGCCCTGTCCAAGGTATCCTTTGAGCCCAATTCCCATACATTATTGT
205 S C N T S V I T Q A C P K V S F E P I P I H Y C

685 GCCCCGGCTGGTTTTCGATTCTAAANTGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTC
229 A P A G F A I L K C N N K T F N G T G P C T N V

757 AGCACAGTACAAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTGAATGGCAGTCTAGCA
253 S T V Q C T H G I R P V V S T Q L L L N G S L A

829 GAAGAAGAGGTAGTAATTAGATCTGCCAATTTACAGACAAATGCTAAACCAATAATAGTACAGCTGAACCAA
277 E E E V V I R S A N F T D N A K T I I V Q L N Q

901 TCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAAACATTAGTAGAGCAAAATGGAATGCCACTTTAAAA
301 S V E I N C T G A G H C N I S R A K W N A T L K

973 CAGATAGCTAGCAAAATTAAGAGAACAAATTTGGAAATAATAAAACAATAATCTTTAAGCAATCCTCAGGAGGG
325 Q I A S K L R E Q F G N N K T I I F K Q S S G G

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FIGURE 10C

```

1045 GACCCAGAAATTGTAACGCACAGTTTAAATTGGAGGGGAATTTTCTACTGTAAATTCACACAACTGTTT
349 D P E I V T H S F N C G G E F F Y C N S T Q L F

1117 AATAGTACTTGGTTTAAATAGTACTTGGAGTACTGAAGGGTCAATAACACTGAAGGAAGTGACACAATCACA
373 N S T W F N S T W S T E G S N N T E G S D T I T

1189 CTCCCATGCAGATAAAACAATTATATAACATGGTGCAGGAAGTAGGAAAAGCAATGTATGCCCTCCCATC
397 L P C R I K Q F I N M V Q E V G K A M Y A P P I

1261 AGCGGACAAATTAGATGTTTCATCAATAATTACAGGGCTGCTATTAAACAAGAGATGGTGGTAATAACAACAAT
421 S G Q I R C S S N I T G L L L T R D G G N N N N

1333 GGTCCGAGATCTTCAGACCCTGGAGGAGGAGATATGAGGGACAATTGGAGAGTGAATTATATAATAATAA
445 G S E I F R P G G G D M R D N W R S E L Y K Y K

1405 GTAGTAAATAATTGAACCATTAGGAGTAGCACCACCAAGGCAAGAGAGAGTGGTGCAGAGAGAAAATGA
469 V V K I E P L G V A P T K A K R R V V Q R E K -
      NotI
1447 GGGGGGGC

```

FIGURE 11A

FIGURE 11A
FIGURE 11B
FIGURE 11C

JR-EL ΔV3-CD4⁻
1
1

19
7

79
27

139
47

199
67

259
87

319
107

379
127

ATGGATGCAATGAAGAGA
M D A M K R

GGGCTCTGCTGTGCTGTGTGGAGCAGTCTTCGTTTCCGCCAGCCAGGAATC
G L C C V L L L C G A V F V S P S Q E I

CATGCCCGATTGAGAGAGCGCGCAGAGTAGAAAGTTGTGGTCCACAGTCTATTATGGG
H A R F R R G A R V E K L W V T V Y Y G

GTACCTGTGTGGAAGAAGCAACCACCCTCTATTTTGTGCATCAGATGCTAAAGCATAT
V P V W K E A T T T L F C A S D A K A Y

GATACAGAGGTACATAATGTTTGGGCCACACATGCCCTGTGTACCCACAGACCCCAACCCA
D T E V H N V W A T H A C V P T D P N P

CAAGAAGTAGTATTGGAATAATGTAACAGAACATTTTAACATGTGGAAAAATAACATGGTA
Q E V V L E N V T E H F N M W K N N M V

GAACAGATGCAGGAGGATATAATCAGTTTATGGGATCAAAGCCCTAAAGCCATGTGTAAAA
E Q M Q E D I I S L W D Q S L K P C V K

TTAACCCCACTCTGTGTACTTTAAATTGCAAGGATGTGAATGCTACTAATACCACCTAAT
L T P L C V T L N C K D V N A T N T T N

NarI

▲ Signal cleavage

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FIGURE 11B

439 GATAGCGAGGGAACGATGGAGAGAGGAGAAATAAAAACTGCTCTTTCAATATCACCACA
 147 D S E G T M E R G E I K N C S F N I T T
 499 AGCATAAGAGATGAGGTGCAGAAAGAATATGCTCTTTTTTATAAACTTGATGTAGTACCA
 167 S I R D E V Q K E Y A L F Y K L D V V P
 559 ATAGATAATAATACCAGCTATAGTTGATAAGTTGTGACACCTCAGTCATTACACAG
 187 I D N N N T S Y R L I S C D T S V I T Q
 619 GCCTGTCCAAAGATATCCTTTGAGCCCAATCCCATACATTATTGTGCCCGCTGTTTT
 207 A C P K I S F E P I P I H Y C A P A G F
 679 GCGATTCTAAAGTGTAATGATAAGACGTTCAATGGAAAGGACCATGTAAAAATGTCAGC
 227 A I L K C N D K T F N G K G P C K N V S
 739 ACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCACTGCTGCTAAATGGC
 247 T V Q C T H G I R P V V S T Q L L L N G
 799 AGCTAGCAGAGAAGAGGTAGTAATTAGATCTGACAATTTCCAGAACAAATGCTAAACC
 267 S L A E E E V V I R S D N F T N N A K T
 859 ATAATAGTACAGCTGAAGAATCTGTAGAAATTAATTGTACAGGTGCTGGACATTGTAAC
 287 I I V Q L K E S V E I N C T G A G H C N
 919 ATTAGTAGAGCAAAATGGAATGACACTTTAAACAGATAGTTATAAAATTAAGAGAACAA
 307 I S R A K W N D T L K Q I V I K L R E Q

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FIGURE 11C

979 TTTGAGAAATAACAATAGTCTTTTAATCACTCCTCAGGAGGGAGCCAGAAATTGTAATG
 327 F E N K T I V F N H S S G G D P E I V M

 1039 CACAGTTTAAATTGTGGAGGAGAAATTTTCTACTGTAAATTCACACACACACTGTTTAATAGT
 347 H S F N C G G E F F Y C N S T Q L F N S

 1099 ACTTGGAAATAATACTGAAGGTCAATAACACTGAAGGAAATATCATCACACTCCCA
 367 T W N N N T E G S N N T E G N T I T L P

 1159 TGCAGAATAAAACAAATTATAAATCATGGTGCAGGAAGTAGGAAAGCAATGTATGCCCT
 387 C R I K Q I I N M V Q E V G K A M Y A P

 1219 CCCATCAGAGGACAAATTAGATGTTTCATCAATATTACAGGGCTGCTATTAAACAAGAGAT
 407 P I R G Q I R C S S N I T G L L L T R D

 1279 GGTGGTATTAAATGAGAAATGGGACCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGGAC
 427 G G I N E N G T E I F R P G G G D M R D

 1339 AATTGGAGAAGTGAATTATATAATAATAAGTAGTAATAAATTGAACCATTAGGAGTAGCA
 447 N W R S E L Y K Y K V V K I E P L G V A

 1399 CCCACCAAGGCAAGAGAAGAGTGGTGCAAAGAGAGAAATGAGCGGCGGC
 487 P T K A K R R V V Q R E K -

NotI

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FIGURE 12A

FIGURE 12A
FIGURE 12B
FIGURE 12C

LAI CD4⁻

1 tPA signal sequence
 1 ATGGATGCAATGAAGAGAGGGCTCTGCTGT
 M D A M K R G L C C

37 GTGCTGCTGTGTGGAGCAGTCTTTCGTTCCGCCAGCCAGGAAATCCATGCCCGATTTCAGAAAGAGCGCC
 13 V L L L C G A V F V S P S Q E I H A R F R R G A
 Nal

109 AGACAGAAATTTGTGGTCCACAGTCTATTATGGGGTACCTGTGTGGAGGAGCAACCACCTCTATT
 37 R T E K L W V T V Y Y G V P V W K E A T T T L F
 ▲ Signal cleavage

181 TGTGCATCAGATGCTAAAGCATATGATACAGAGGTACATAATGTTTGGGCCACACATGCCCTGTGTACCCACA
 61 C A S D A K A Y D T E V H N V W A T H A C V P T

253 GACCCCAACCCACAAGTAGTATTGGTAAATGTGACAGAAATTTTAAATGTGGAAATGACATGGTA
 85 D P N P Q E V V L V N V T E N F N M W K N D M V

325 GAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAGCCCTAAAGCCATGTGTAAATTAACCCCACTC
 109 E Q M H E D I I S L W D Q S L K P C V K L T P L

397 TGTGTTAGTTTAAAGTGCACCTGATTGGGGGAATGCTACTAATACCAATAGTAGTAGC
 133 C V S L K C T D L G N A T N T N S S N T N S S S

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FIGURE 12B

469 GGGGAATGATGGAGAAAGGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAAGCATAAGAGGTAAG
157 G E M M E K G E I K N C S F N I S T S I R G K

541 GTGCAGAAAGAATATGCATTTTTTATAAACTTGATATAATACCAATAGATAATGATACTACCAGCTATACG
181 V Q K E Y A F F Y K L D I I P I D N D T T S Y T

613 TTGACAAGTTGTACACCTCAGTCATTACACAGGCCCTGTCCAAGGTATCCTTTGAGCCAAATTCCCATACAT
205 L T S C N T S V I T Q A C P K V S F E P I P I H

685 TATTGTGCCCCGGCTGGTTTTCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACA
229 Y C A P A G F A I L K C N N K T F N G T G P C T

757 AATGTCAGCACAGTACAATGTACACATGGAAATTAGGCCAGTAGTATCAACTCAACTGCTGTGTAATGCGAGT
253 N V S T V Q C T H G I R P V V S T Q L L L N G S

829 CTAGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTCCACAGACAATGCTAAACCATAATAGTACAGCTG
277 L A E E E V V I R S A N F T D N A K T I I V Q L

901 AACCAATCTGTAGAAATTAATTGTACAGACCCCAACAATAACAAGAAAGTATCCGTATCCAGAGGGGA
301 N Q S V E I N C T R P N N N T R K S I R I Q R G

973 CCAGGGAGAGCATTGTGTACAAATAGGAAAAATAGGAAATATGAGACAAGCACATTGTAAACATTAGTAGGCA
325 P G R A F V T I G K I G N M R Q A H C N I S R A

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FIGURE 12C

1045 AAATGGAATGCCACTTTAAACAGATAGCTAGCAAAATTAAGAGAGAACAAATTGGAAATAATAAACAAATAATC
 349 K W N A T L K Q I A S K L R E Q F G N N K T I I

 1117 TTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTAAATTGTGGAGGGGAATTTTCTAC
 373 F K Q S S G G D P E I V T H S F N C G G E F F Y

 1189 TGTAAATCAACACAACCTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTGAAGGGTCAATAACACT
 397 C N S T Q L F N S T W F N S T W S T E G S N N T

 1261 GAAGGAAGTGACACAATCACACTCCCATGCAGAAATAAAACAATTATATAACATGGTGAGGAAGTAGGAAAA
 421 E G S D T I T L P C R I K Q F I N M V Q E V G K

 1333 GCAATGTATGCCCTCCCATCAGCGGACAAATTAGATGTTTCATCAATAATTACAGGGCTGCTATTAAACAAGA
 445 A M Y A P P I S G Q I R C S S N I T G L L L T R

 1405 GATGGTGGTAATAACAACAATGGGTCCGAGATCTTCAGACCTGGAGGAGGAGATATGAGGACAATTGGAGA
 469 D G G N N N N G S E I F R P G G G D M R D N W R

 1477 AGTGAATTATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAGAGAAGA
 493 S E L Y K Y K V V K I E P L G V A P T K A K R R

 1549 GTGGTGCAGAGAGAAAATGAGCGGCGGC
 517 V V Q R E K -

NotI

FIGURE 13A

FIGURE 13A
FIGURE 13B
FIGURE 13C
FIGURE 13D

JR-FL CD4'

1	ATGGATGCAATGAAGAGA	
1	M D A M K R	
19	GGGCTCTGCTGTGCTGTGCTGTGGAGCAGTCTTCGTTTCGCCAGCCAGGAATC	
7	G L C C V L L L C G A V F V S P S Q E I	
	NarI	
79	CATGCCCGATTGAGAGAGGGCGGAGTAGAAAGTTGTGGGTCACAGTCTATTATGGG	
27	H A R F R R G A R V E K L N V T V Y Y G	
	▲ Signal cleavage	
139	GTACCTGTGTGGAAGAACCAACCACCTCTATTTTGTGCATCAGATGCTAAAGCATAT	
47	V P V W K E A T T T L F C A S D A K A Y	
199	GTACAGAGGTACATAATGTTTGGGCCACACATGCTGTGTACCCACAGACCCCAACCCA	
67	D T E V H N V W A T H A C V P T D P N P	
259	CAAGAAGTAGTATTGGAATAATGTAACAGAACATTTTAACATGTGGAAATAACATGGTA	
87	Q E V V L E N V T E H F N M W K N N M V	

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FIGURE 13B

319 GAACAGATGCAGGAGGATATAATCAGTTTATGGATCAAGCCTAAAGCCATGTGTAAA
107 E Q M Q E D I I S L W D Q S L K P C V K

379 TTAACCCCACTCTGTGTACTTTAATTGCAAGGATGTGAATGCTACTAATACCCTAAT
127 L T P L C V T L N C K D V N A T N T T N

439 GATAGCGAGGGAACGATGGAGAGGAGGAATAAAACTGCTCTTTCAATATCACCACA
147 D S E G T M E R G E I K N C S F N I T T

499 AGCATAAGAGATGAGGTGCAGAAAGAAATATGCTCTTTTATAAACTTGATGTAGTACCA
167 S I R D E V Q K E Y A L F Y K L D V V P

559 ATAGATAATAATAACCAAGCTATAGTTGATAAGTTGTGACACCTCAGTCATTACACAG
187 I D N N N T S Y R L I S C D T S V I T Q

619 GCCTGTCCAAGATATCCTTTGAGCCCAATCCCATACATTATTGTGCCCGGCTGGTTT
207 A C P K I S F E P I P I H Y C A P A G F

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FIGURE 13C

679	CGGATTCTAAAGTGTAAATGATAAGACGTTCAATGGAAAGGACCATGTAAAAATGTCAGC
227	A I L K C N D K T F N G K G P C K N V S
739	ACAGTACAATGTACACATGGGAATTAGGCCAGTAGTATCAACTCAACTGCTGCTAAATGGC
247	T V Q C T H G I R P V V S T Q L L L N G
799	AGTCTAGCAGGAAGAGGAGTAGTAATTAGATCTGACAATTCACGAACAATGCTAAACCC
267	S L A E E V I R S D N F T N N A K T
859	ATAATAGTACAGCTGAAGAATCTGTAGAAATTAATTGTACAAGACCCCAACAATACA
287	I I V Q L K E S V E I N C T R P N N T
919	AGLAAAGTATACATATAGGACCAGGAGAGCATTTTATACAGGAGAAATATAGGA
307	R K S I H I G P G R A F Y T T G E I I G
979	GATATAAGACAAGCACATTGTACATTAGTAGAGCAAAATGGAATGACACTTTAAACAG
327	D I R Q A H C N I S R A K W N D T L K Q

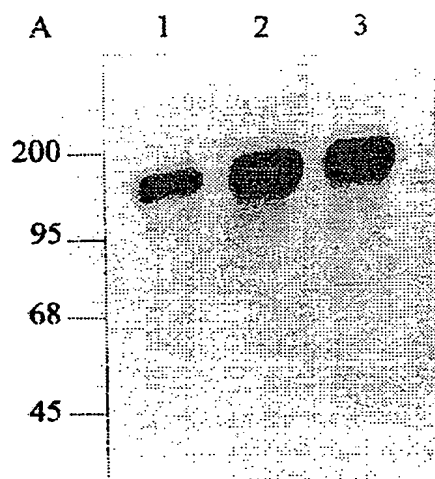
34/42

FIGURE 13D

1039	ATAGTTATAAAATTAAGAGAACAAATTGAGAAATAAAACAATAGTCTTTAATCACTCCTCA
347	I V I K L R E Q F E N K T I V F N H S S
1099	GGAGGGACCCAGAAATTGTAATGCACAGTTTAAATTGTGGAGGAGAAATTTTCTACTGT
367	G G D P E I V M H S F N C G G E F F Y C
1159	AATTCAACACAACACTGTTTAAATAGTACTTGGAAATAATACTGAAGGGTCAATAACACT
387	N S T Q L F N S T W N N N T E G S N N T
1219	GAAGGAAATACATCACTCCCATGCAGAAATAAAACAATAATTATAACATGGTGCAGGAA
407	E G N T I T L P C R I K Q I I N M V Q E
1279	GTAGGAAAGCAATGTATGCCCTCCCATCAGAGGACAATAATTAGATGTTTCATCAATATT
427	V G K A M Y A P P I R G Q I R C S S N I
1339	ACAGGGCTGCTATTAAACAAGAGATGGTGGTATTAAATGAGAATGGACCGAGATCTTCAGA
447	T G L L L T R D G G I N E N G T E I F R
1399	CCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAATAAGTAGTA
467	P G G G D M R D N W R S E L Y K Y K V V
1459	AAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAGAGAGAGAGTGTTGCGAAAGAGAA
487	K I E P L G V A P T K A K R R V V Q R E
	NotI
1519	AAATGAGCGGCGCG
507	K

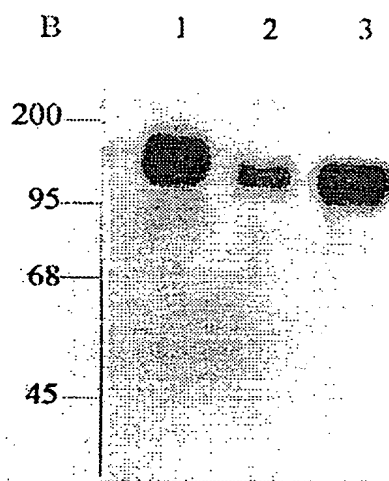
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FIGURE 14A



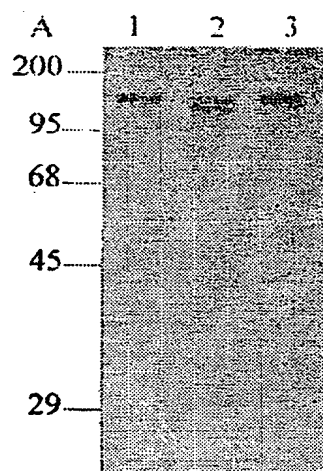
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FIGURE 14B



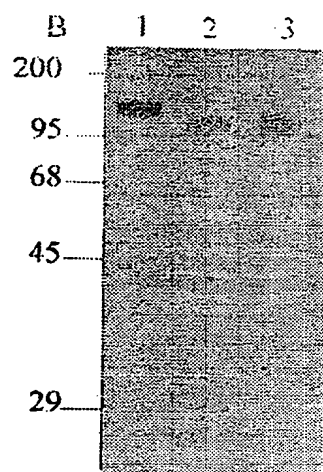
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FIGURE 15A



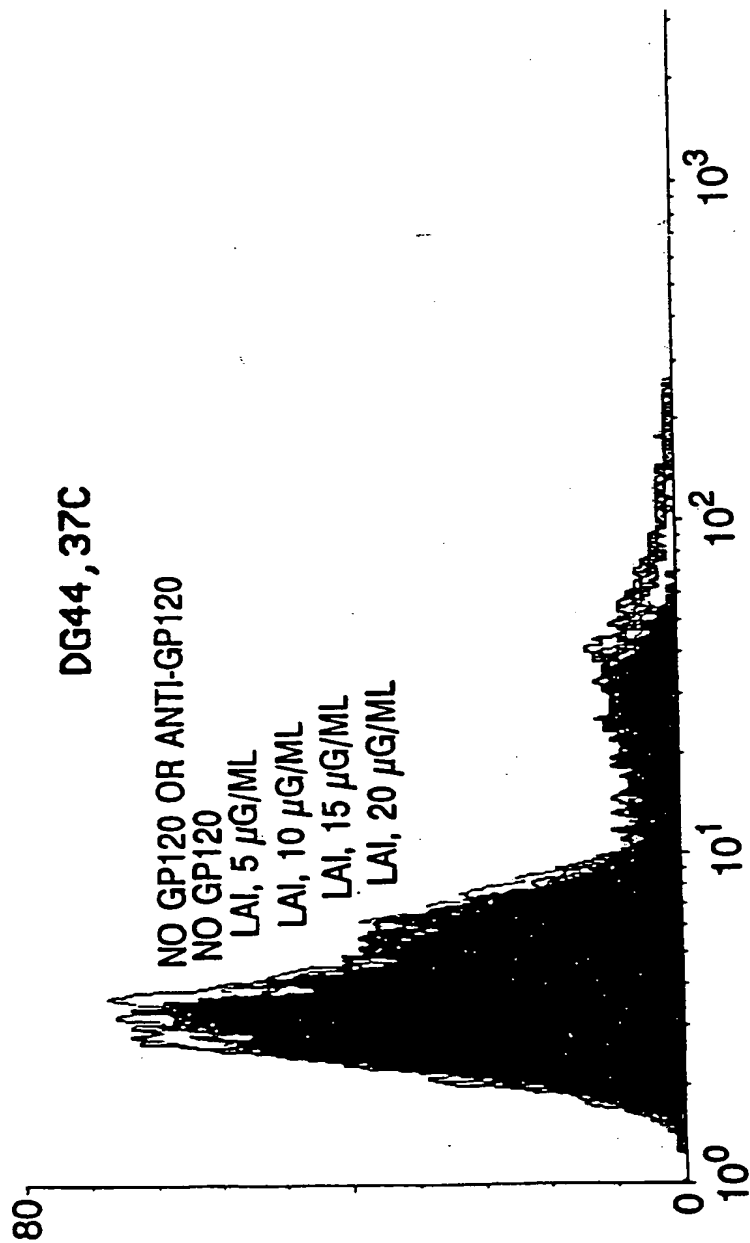
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FIGURE 15B



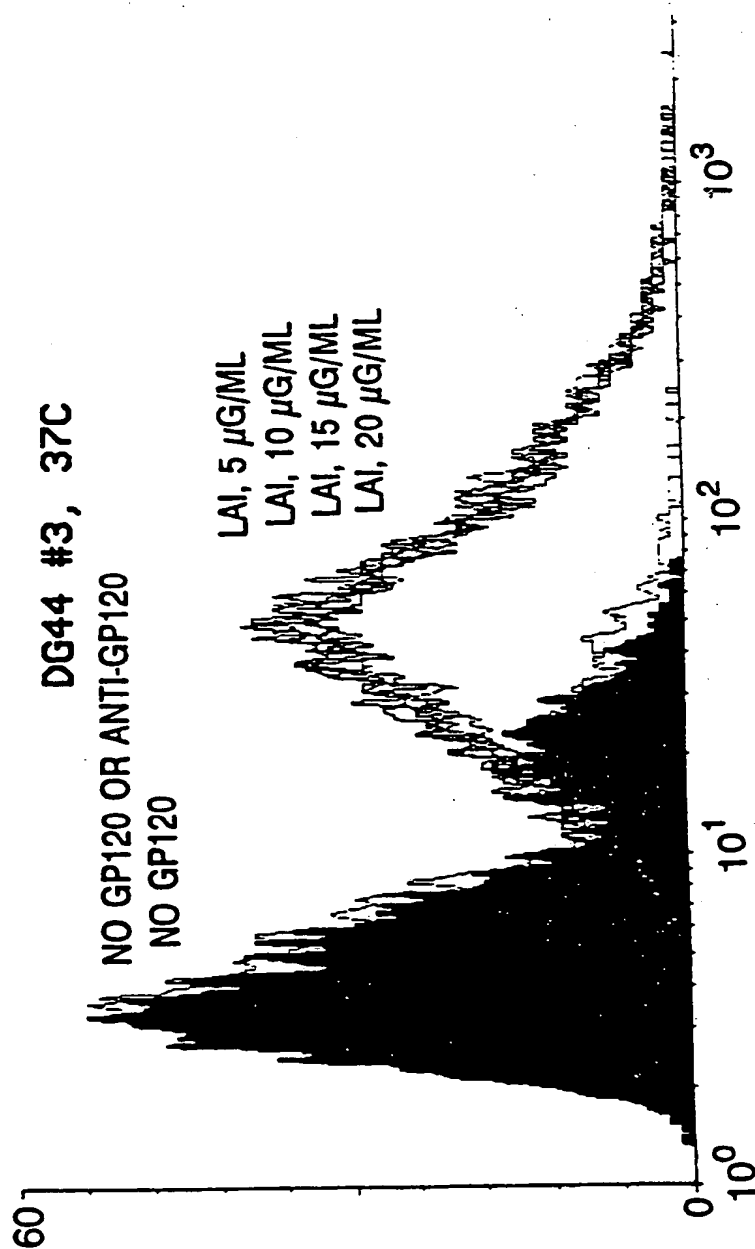
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FIGURE 16A



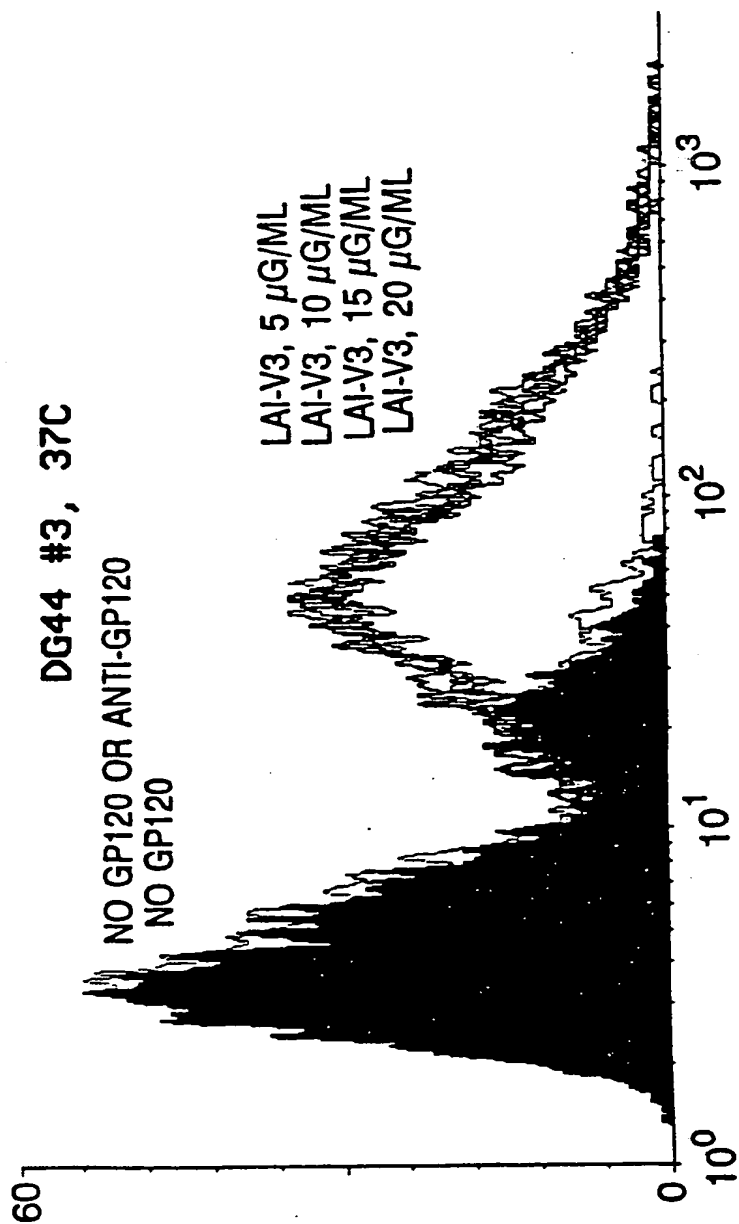
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FIGURE 16B



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FIGURE 16C

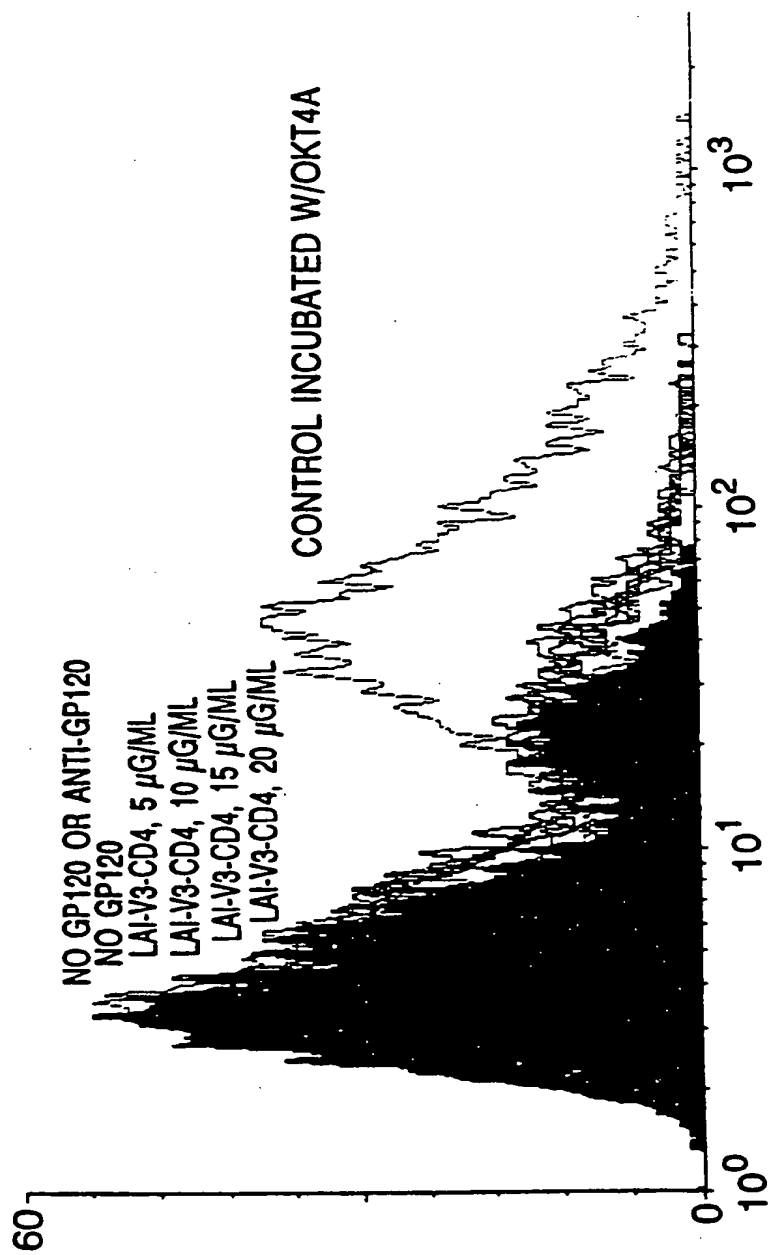


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FIGURE 16D

DG44 #3, 37C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/03282

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 424/88, 89; 536/27; 530/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 536/27; 530/395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms: HIV-1, mutation, V3 loop, C4 region, envelope glycoprotein, vaccines, nucleic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 252, issued 17 May 1991, S. Wain-Hobson, et al, "LAV Revisited: Origins of the Early HIV-1 Isolates from Institut Pasteur", pages 961-965, see entire article.	6,7
Y	US, A, 5,030,449 (BERZOFKY ET AL) 09 July 1991, cols. 4-7.	1-27
Y	WO, A, 91/15512 (GREGORY ET AL) 17 October 1991, entire patent.	1-27
Y	WO, A, 91/11461 (PASEK ET AL) 08 August 1991, see entire patent.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

09 JUNE 1994

Date of mailing of the international search report

JUN 24 1994

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/03282

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61K 39/12, 39/00; C07K 17/00, 3/00, 13/00, 15/00; C07H 15/12, 13/00